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Kipps et al.

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(54) **THERAPEUTIC ANTIBODIES AGAINST ROR-1 PROTEIN AND METHODS FOR USE OF SAME**

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(73) Assignee: **The Regents of the University of California**, Oakland, CA (US)

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§ 371 (c)(1),
(2), (4) Date: **Jun. 27, 2013**

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PCT Pub. Date: **Jul. 19, 2012**

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(51) **Int. Cl.**

C07K 16/40	(2006.01)
C07K 16/28	(2006.01)
A61K 39/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C07K 16/40*** (2013.01); ***C07K 16/2803*** (2013.01); *A61K 2039/505* (2013.01); *C07K 2317/565* (2013.01); *C07K 2317/73* (2013.01); *C07K 2317/77* (2013.01); *C07K 2317/92* (2013.01)

(58) **Field of Classification Search**

CPC C07K 16/40; C07K 16/2803; C07K 2317/565; C07K 2317/73; C07K 2317/77; A61K 2039/505

See application file for complete search history.

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Extended European Search Report Regarding EP 12 734 733.4.

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Primary Examiner — Ruixiang Li

(74) *Attorney, Agent, or Firm* — Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C.

(57) **ABSTRACT**

Therapeutic antibodies having binding specificity for ROR-1 expressed on cancer cells (particularly leukemic and lymphomic cells) and pharmaceutical compositions containing one or more such antibodies for use in treating cancer. Methods for diagnosing such cancers through in vitro detection of binding to ROR-1 protein expressed on putative cancer cells are also provided.

9 Claims, 25 Drawing Sheets

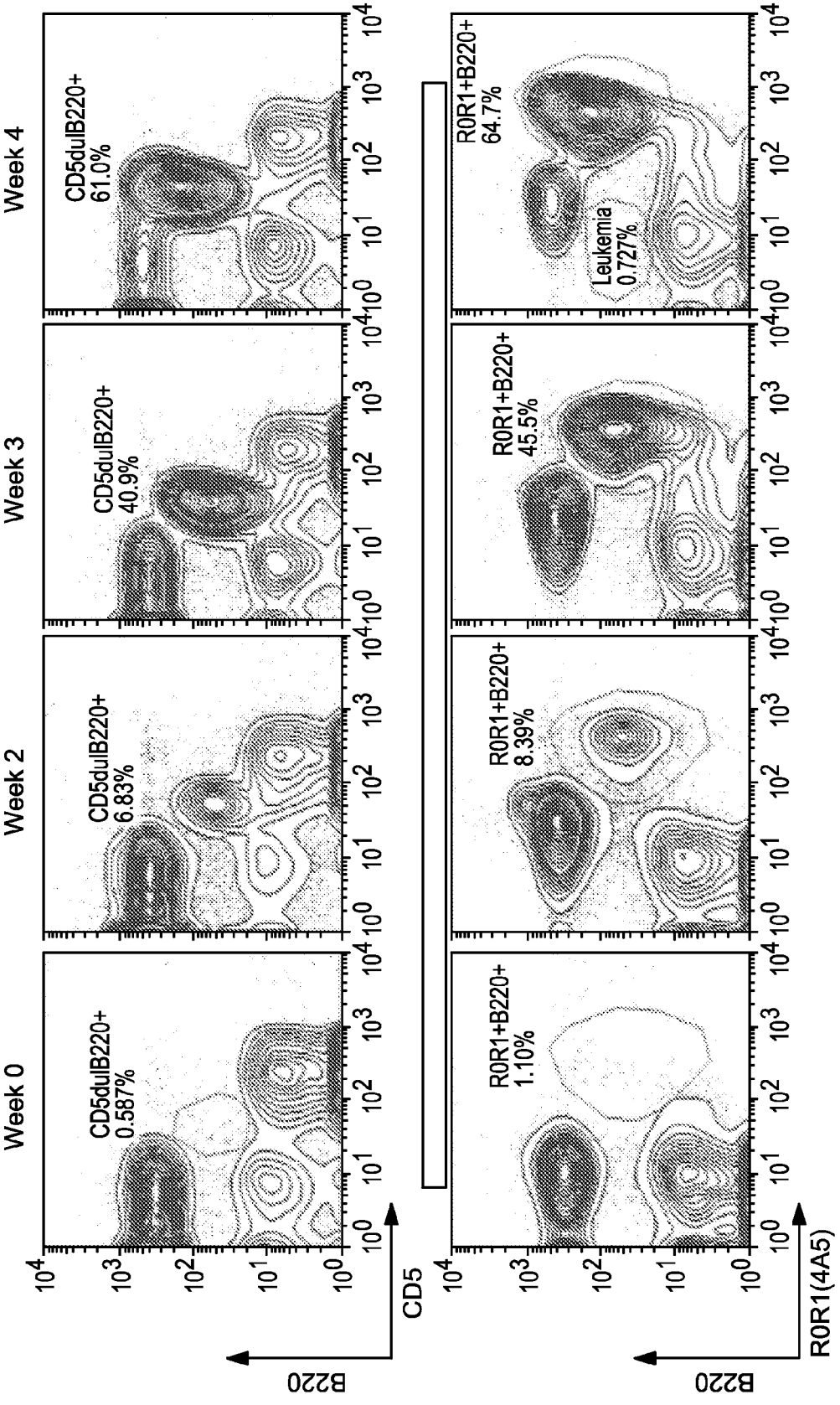

Week 0
Week 2
Week 3
Week 4
CD5dulB220+
0.587%
CD5dulB220+
6.83%
CD5dulB220+
40.9%
CD5dulB220+
61.0%
R0R1+B220+
1.10%
R0R1+B220+
8.39%
R0R1+B220+
45.5%
R0R1+B220+
64.7%
Leukemia
0.727%
B220
CD5
B220
R0R1(4A5)

FIG. 1

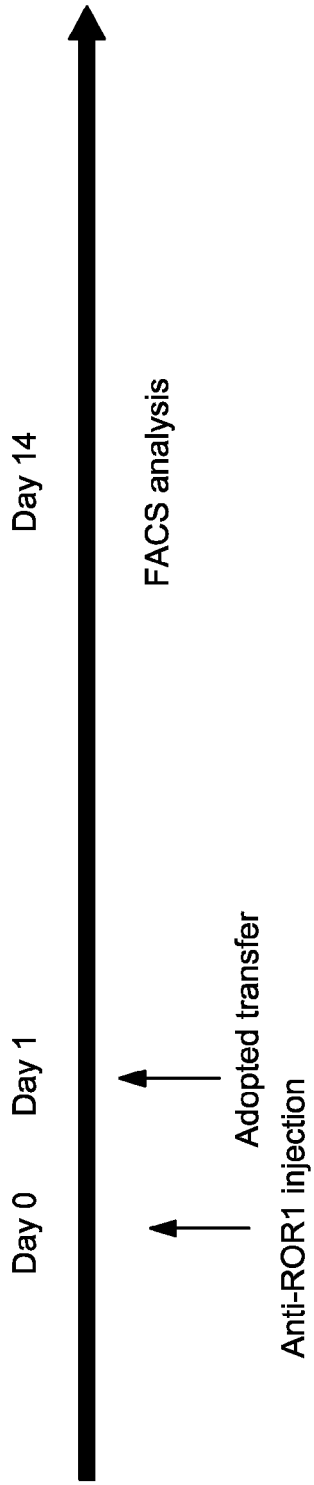

Day 0
Day 1
Day 14
Anti-ROR1 injection
Adopted transfer
FACS analysis

FIG. 2

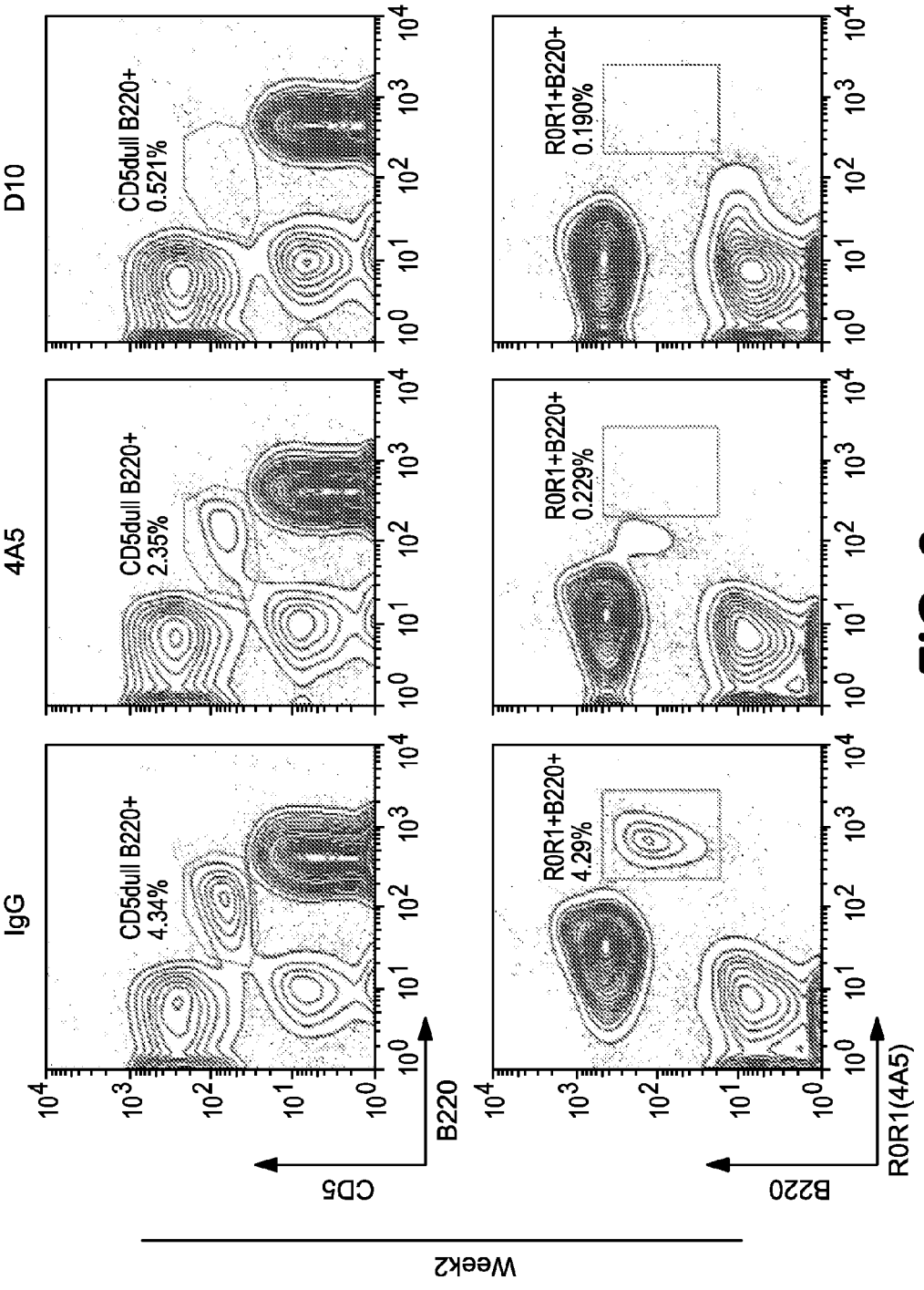

IgG
4A5
D10
CD5dull B220+
4.34%
CD5dull B220+
2.35%
CD5dull B220+
0.521%
R0R1+B220+
4.29%
R0R1+B220+
0.229%
R0R1+B220+
0.190%
CD5
B220
B220
R0R1(4A5)
Week2

FIG. 3

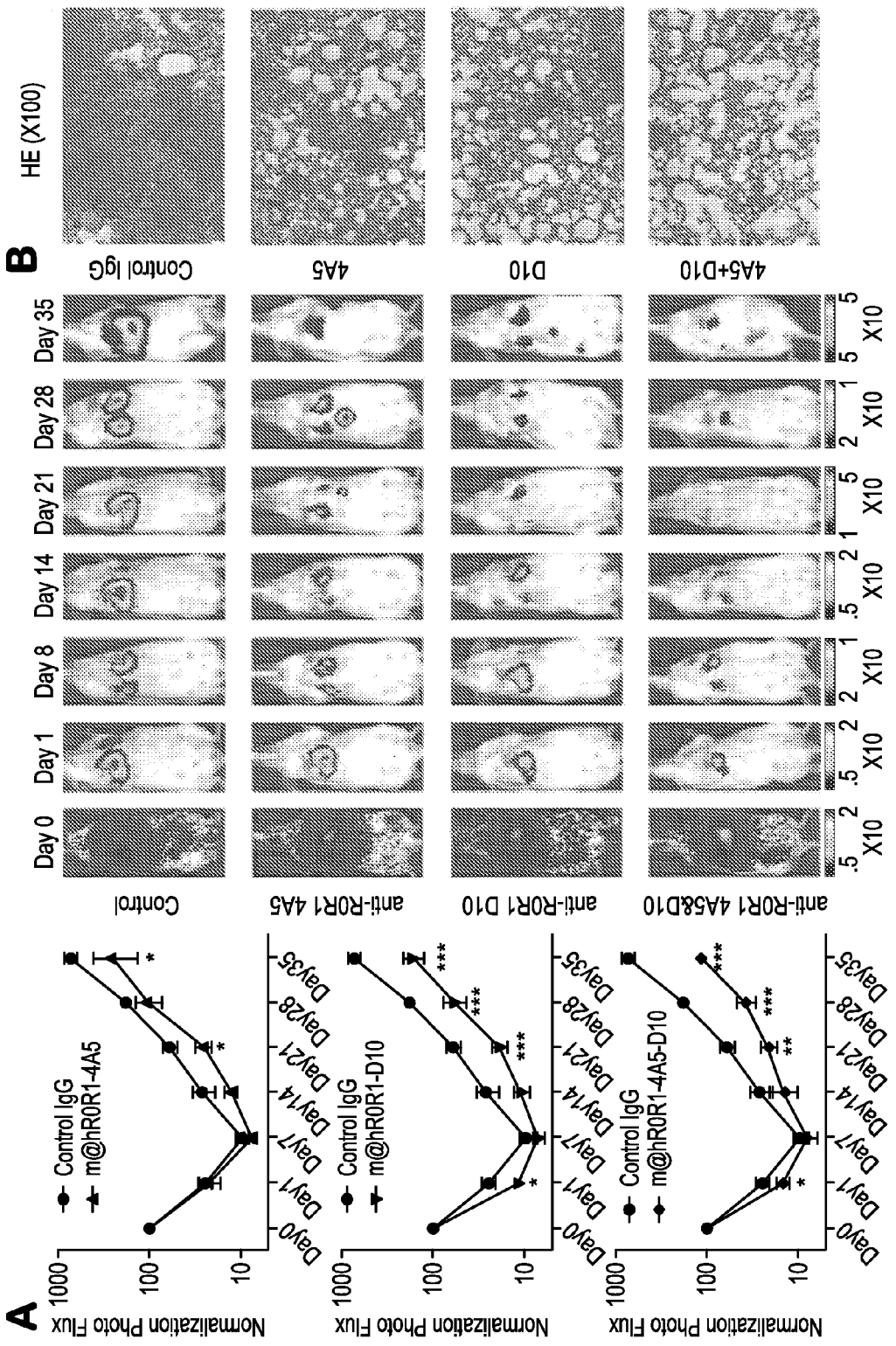

A
Normalization Photo Flux
Control IgG
m@hR0R1-4A5
m@hR0R1-D10
Day0
Day1
Day7
Day14
Day21
Day28
Day35
B
HE (X100)
Control IgG
4A5
D10
4A5+D10
Control
anti-R0R1 4A5
anti-R0R1 D10
anti-R0R1 4A5&D10
Day 0
Day 1
Day 8
Day 14
Day 21
Day 28
Day 35
X10

FIG. 4

Comparison of 4A5 Ig Heavy Chain to The Closest Germline Mouse and Human IGHV

	FR1-IMGT (1-26)			CDR1-IMGT (27-38)	FR2-IMGT (39-55)		
	1	10	20	30	40	50	
4A5_VH	EVKLVESGG.	GLVKPGGSLKLSCAAS		GFTF....SSYA	MSWVRQIPEKRLEWVAS		
MuIGHV5-09*02	.....	.....	.....	..A.....D	.....T.....T		
HuIGHV3-48*01	..Q.....	Q.....R.....		.....S	.N....A.G.G....SY		

	CDR2-IMGT (56-65)	FR3-IMGT (66-104)				
	60	70	80	90	100	
4A5_VH	ISRG...GTT	YYPDSVK.GRFTISRDNVRNILYLQMSSLRSEDTAMYYCGR				
MuIGHV5-09*02	..S....SY.	.....A..T.....L...A.				
HuIGHV3-48*01	..SS..SS.I	..A.....AK.S.....N...A....V...A.				

FIG. 5

Comparison of 2-G6 Ig Heavy Chain to The Closest Germline Mouse and Human IGHV

	FR1-IMGT (1-26)			CDR1-IMGT (27-38)		FR2-IMGT (39-55)		
	1	10	20	30		40	50	
2-G6_QED_VH	EVQLQQSGP.	ELEKPGASVKISCKAS		GFAF....TGYN		MNWVKQTNGKSLEWIGS		
MuIGHV1-39*01	.F.....	.....V.....		.YS.....D..		.....S.....		V
HuIGHV1-02*02	Q...V...A..	VK.....V.....		.YT.....Y		.H..R.AP.QG...M.W		

	CDR2-IMGT (56-65)		FR3-IMGT (66-104)				
	60		70	80	90	100	
G6_QED_VH	IDPY..YGGS		TYNQKFK.DKATLTVDKSSSTAYMQLKSLTSDDSAVYYCAR				
MuIGHV1-39*01	.N.N..	TT	S.....G.....Q.....N....S.....				
HuIGHV1-02*02	.N.N..S..T		N.A...Q.GRV.M.R.T.I.....E.SR.R...T.....				

FIG. 6

Comparison of 2-G3 Ig Heavy Chain to The Closest Germline Mouse and Human IGHV

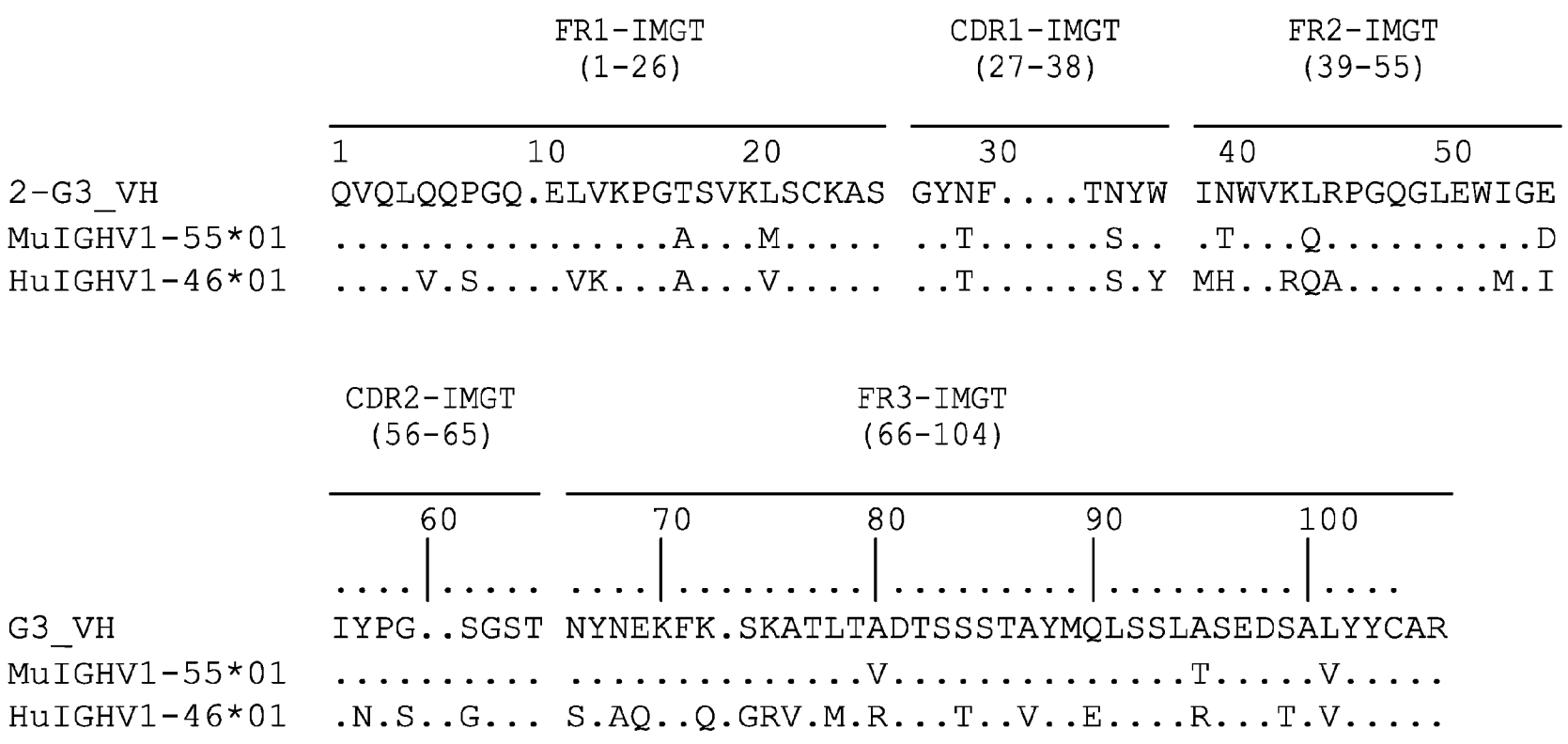


FIG. 7

Comparison of 3-H10 Ig Heavy Chain to The Closest Germline Mouse and Human IGHV

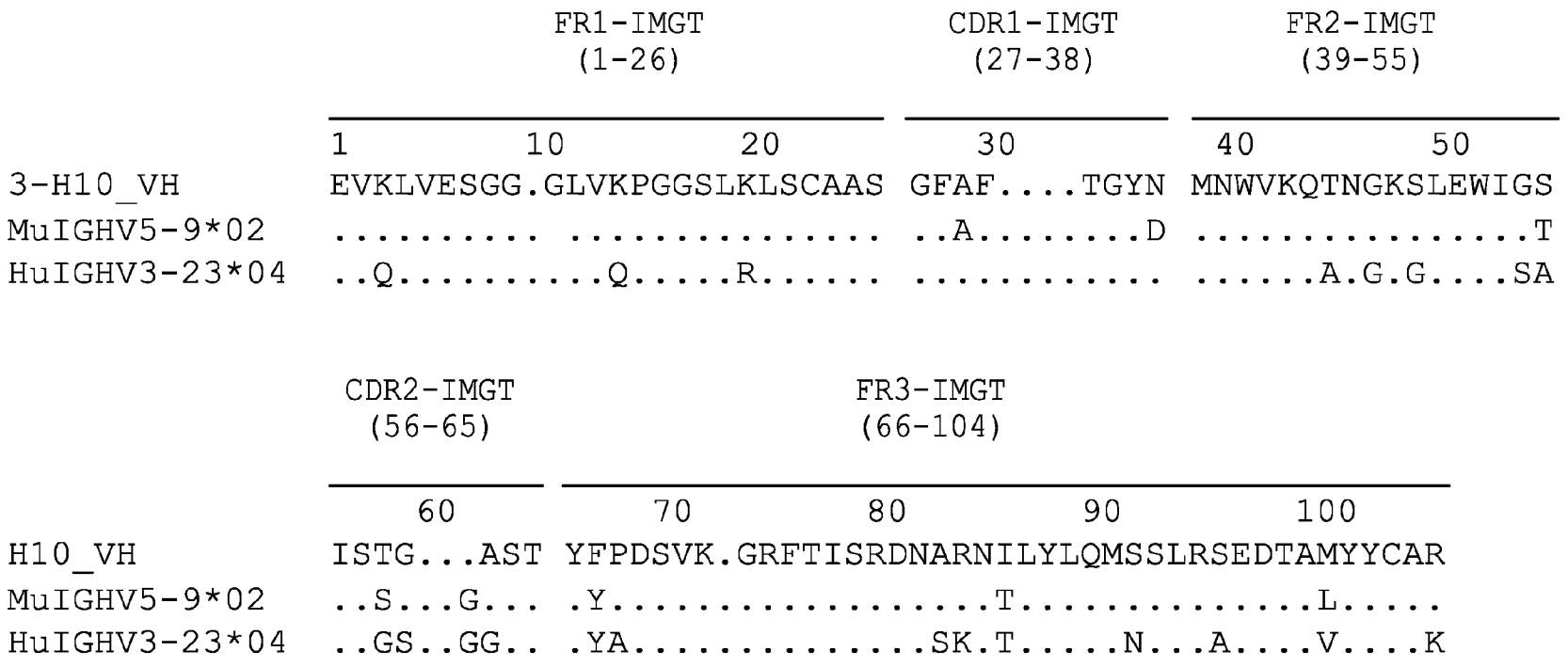


FIG. 8

Comparison of 3-D10 Ig Heavy Chain to The Closest Germline Mouse and Human IGHV

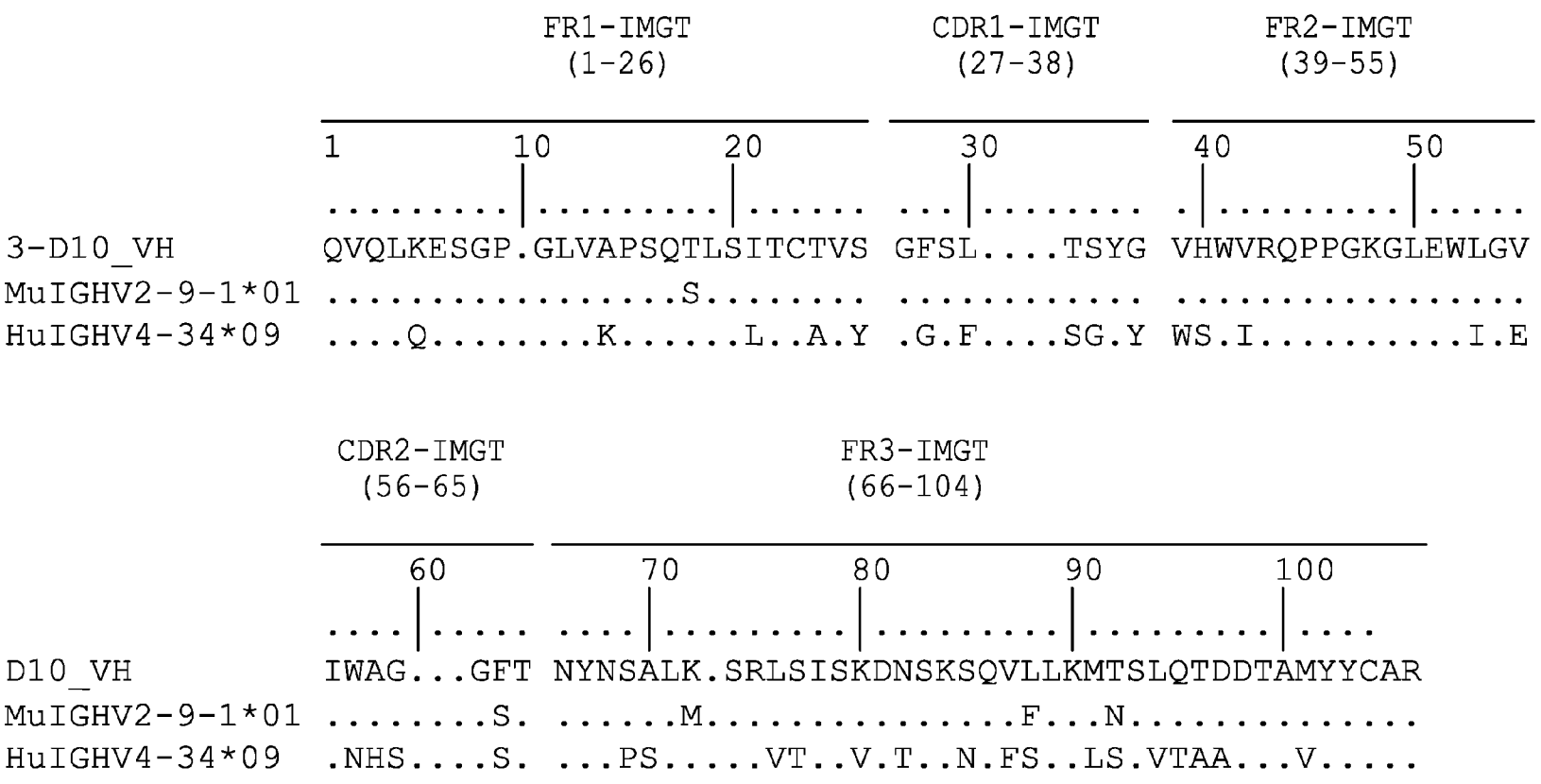
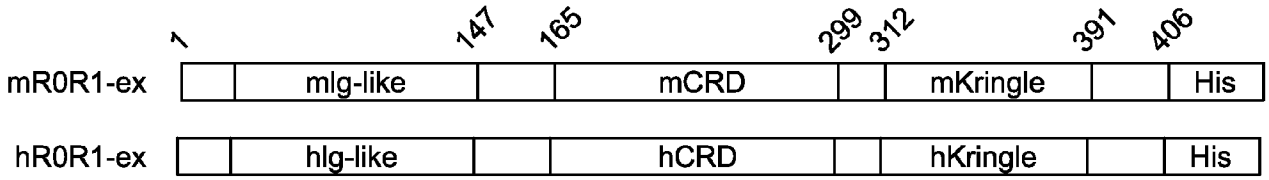


FIG. 9

Human and Murine R0R1 Proteins are Highly Conserved



Region	Position	# of different aa
Ig-like	1---147aa	12
Linker between Ig-like and CRD	148---165aa	1
CRD	166---299aa	1
Linker between CRD and Kringle	300---312aa	0
Kringle	313---391aa	1
Linker between Kringle and TM	392---406aa	0

FIG. 10

Domain Structure and Sequence Homology
of Human and Murine R0R1 Extracellular Protein

```
hR0R1  MHRPRRRGTRPPLLALLAALLLAARGAAAQETELSVSAELVPTSSWNISSELNKDSYLTLDEPMNNITTSLGQTAELHCK 80
mR0R1  ............P.............D.................T...ID.G.....................

                              Ig Domain
hR0R1  VSGNPPPTIRWFKNDAPVVQEPRRLSFRSTIYGSRLRIRNLDTTDTGYFQCVATNGKEVVSSTGVLFVKFGPPPTASPGY 160
mR0R1  .......S..............I...A.N.......................K...T..............S

                                 Cysteine Rich Domain
hR0R1  SDEYEEDGFCQPYRGIACARFIGNRTVYMESLHMQGEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCHYAFPYCDETSS 240
mR0R1  ................................................................................

hR0R1  VPKPRDLCRDECEILENVLCQTEYIFARSNPMILMRLKLPNCEDLPQPESPEAANCIRIGIPMADPINKNHKCYNSTGVD 320
mR0R1  ............V.....................................................................

                                        Kringle
hR0R1  YRGTVSVTKSGRQCQPWNSQYPHTHTFTALRFPELNGGHSYCRNPGNQKEAPWCFTLDENFKSDLCDIPACDSKDSKEKN 400
mR0R1  ....................S...........................................................

hR0R1  KMEILY
mR0R1  ......
```

FIG. 11

Anti-R0R1 Mabs Generated Across Extracellular Domain

	Binding sites of antibodies				
No.	5'-Ig-like	Middle of Ig-like	3'-Ig-like	CRD	Kringle
1-4A5		✓			
G11		✓			
H11		✓			
2G3		✓			
3-D10			✓		

FIG. 12

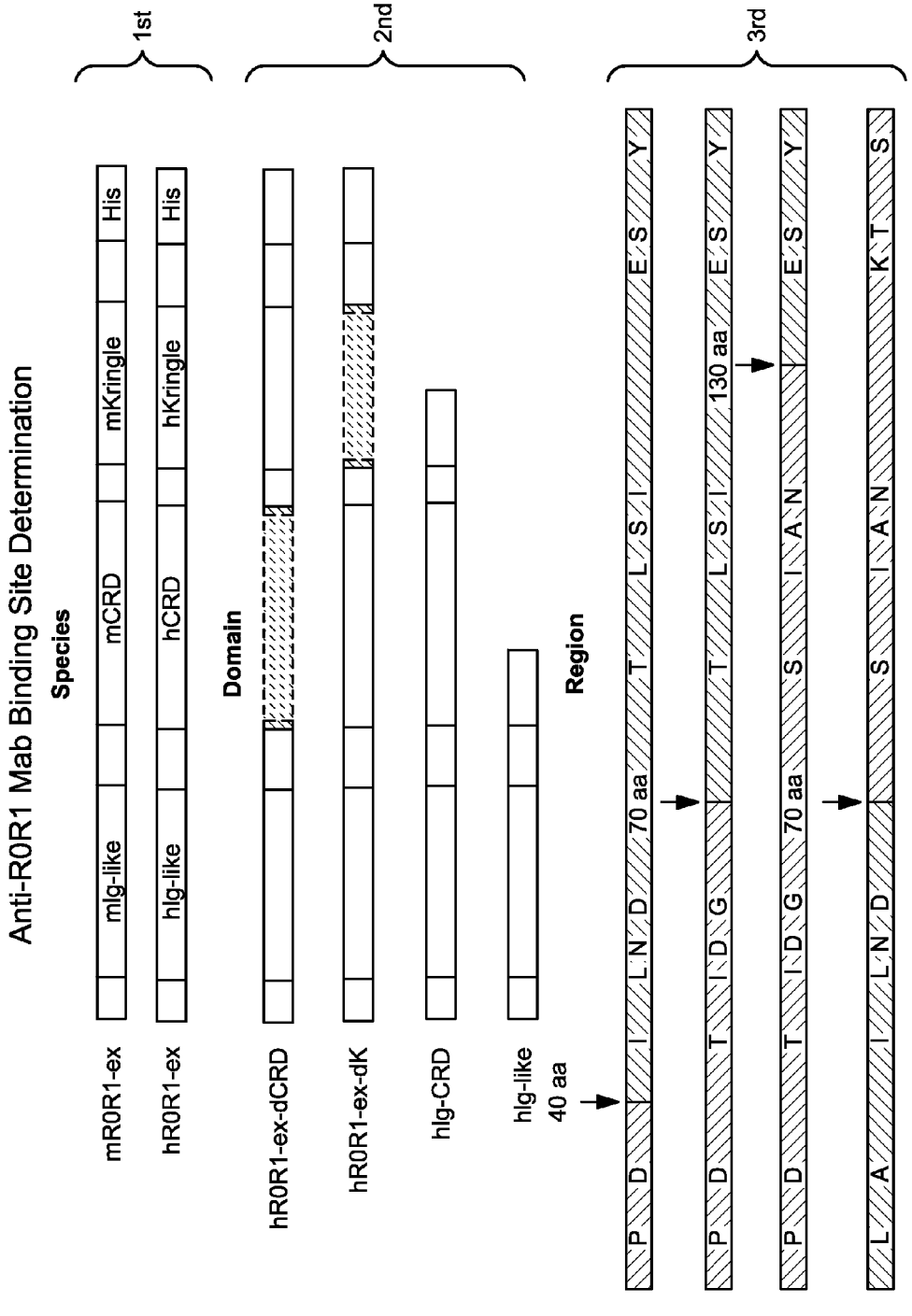

Anti-R0R1 Mab Binding Site Determination
Species
Domain
Region
1st
2nd
3rd
mR0R1-ex
hR0R1-ex
hR0R1-ex-dCRD
hR0R1-ex-dK
hIg-CRD
hIg-like
40 aa
mIg-like
mCRD
mKringle
His
hIg-like
hCRD
hKringle
His
70 aa
130 aa
70 aa
mIg-like
hIg-like

FIG. 13

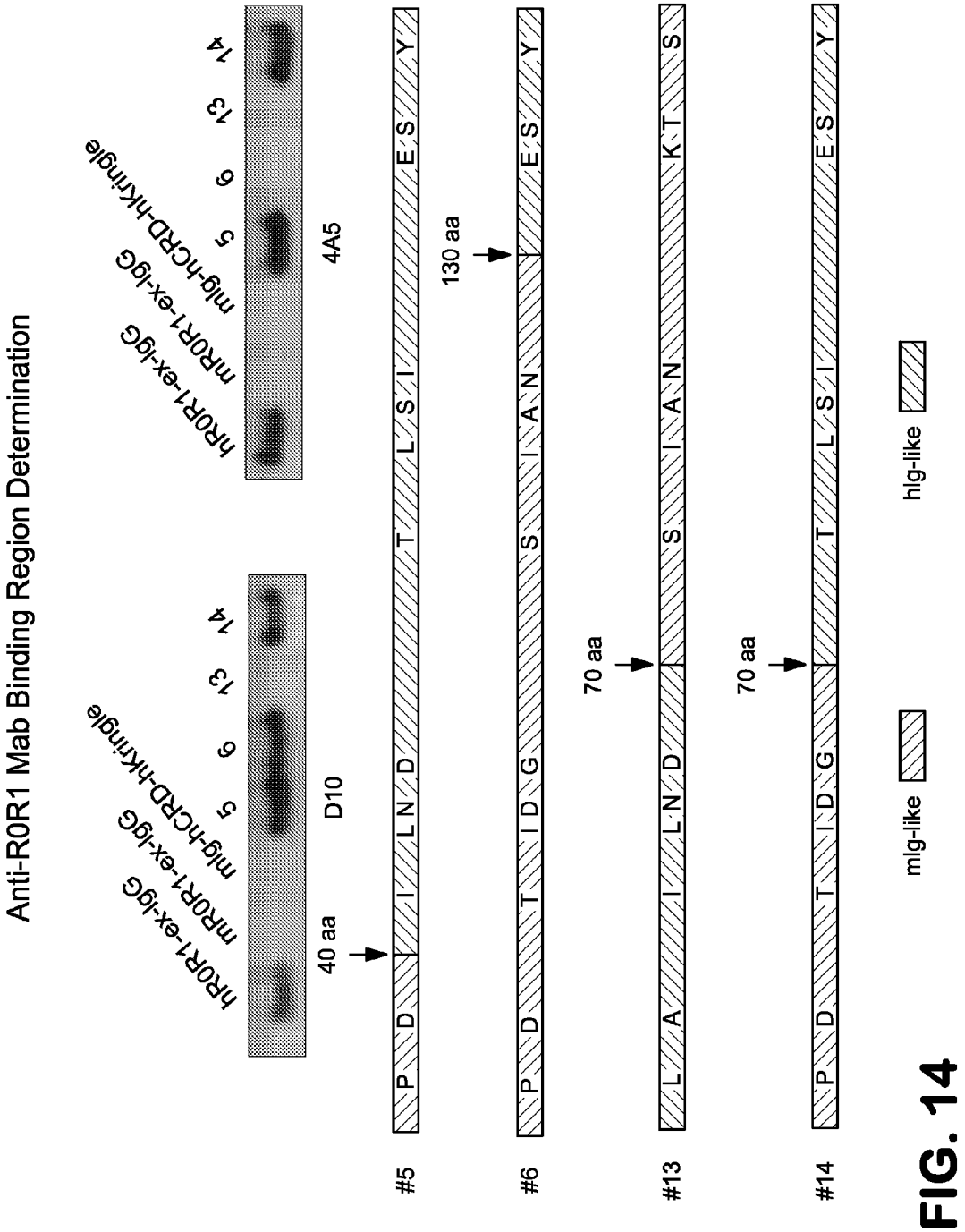
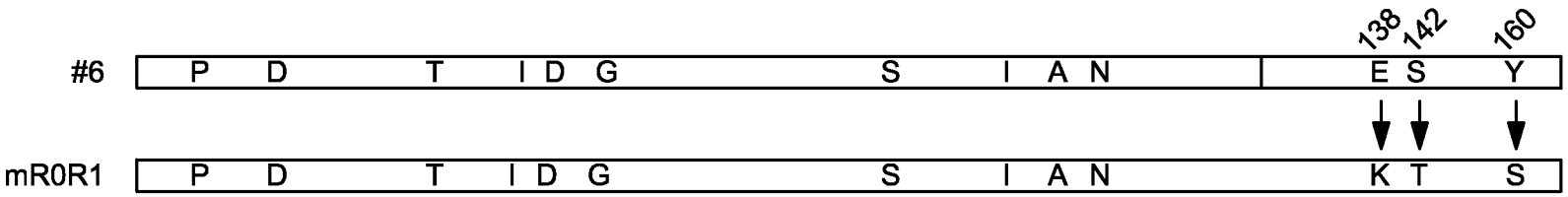
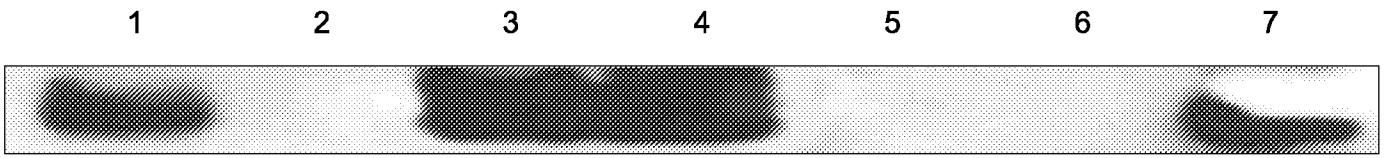

Anti-R0R1 Mab Binding Region Determination
hR0R1-ex-IgG
mR0R1-ex-IgG
mIg-hCRD-hKringle
14
13
6
5
4A5
D10
40 aa
130 aa
70 aa
70 aa
#5
#6
#13
#14
mIg-like
hIg-like

FIG. 14

3-D-10 Binds to the Human Glutamic Acid Residue



138, 142 and 160 amino acids were mutated individually and doubly.



1. #6 protein (wild type)
2. 138 mutation
3. 142 mutation
4. 160 mutation
5. 138-142 mutation
6. 138-160 mutation
7. 142-160 mutation

FIG. 15

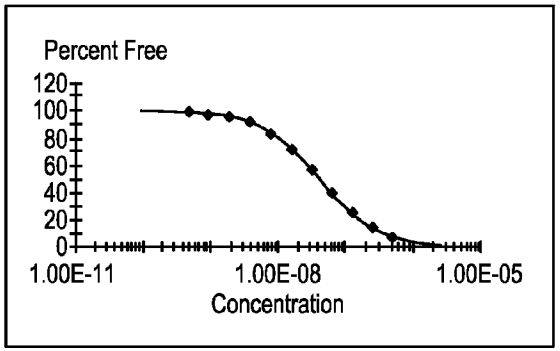
3-D10 Kd Determination

Analysis(x)

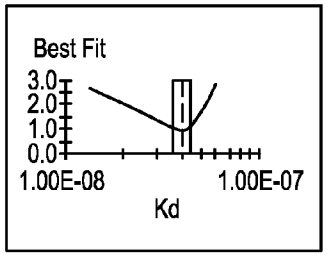
Baseline / Endpoints:
5 to 10 [sec] from beginning
10 to 5 [sec] from end

Ignore	Binding Signal (V)	Concentration
	0.0499	500nM
	0.0805	250nM
	0.1525	125nM
	0.2084	62.5nM
	0.2405	31.25nM
	0.4173	15.63nM
	0.4760	7.81nM
	0.5175	3.90nM
	0.5015	1.95nM
	0.5390	976.56pM
	0.5496	488.28pM
	0.5581	0
✓	0.0012	0
	0.0082	500nM
	0.0777	250nM
✓	0.1245	125nM
✓	0.1684	62.5nM
	0.2908	31.25nM
	0.3642	15.63nM
	0.4170	7.81nM
	04595	3.90nM
	0.4782	1.95nM
	0.4804	976.56pM
	0.4870	488.28pM
	0.4968	0
✓	0.0049	0

Kd = 40.47nM
ABC = 40.47pM
Ratio = 0.0010
Sig 100% = 0.54
Drift = 0.7514
(%/run)
NSB = 0.00
Drift = -0.7086
(mV/run)
%Error = 0.92



Kd = 40.47nM
95% confidence interval
Kd High = 43.50nM
Kd Low = 26.25nM



ABC = 40.47pM
95% confidence interval
ABC High = 0.70nM
ABC Low = Less than 146.19fM

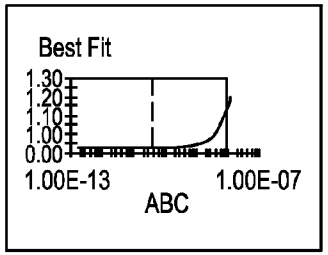


FIG. 16A

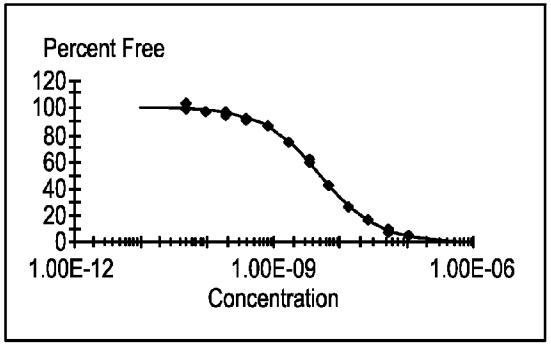
1-4A5 Kd Determination

Analysis(x)

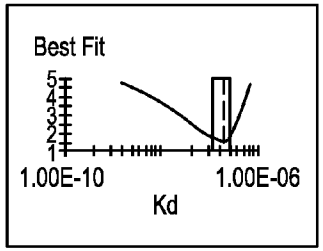
Baseline / Endpoints:
0 to 10 [sec] from beginning
10 to 5 [sec] from end

Ignore	Binding Signal (V)	Concentration
	0.0781	100nM
	0.0990	50nM
	0.1213	25nM
	0.1554	12.5nM
	0.2060	6.25nM
	0.2768	3.13nM
	0.3167	1.56nM
	0.3613	781.25pM
	0.3702	390.63pM
	0.3790	195.31pM
	0.3895	97.66pM
	0.4102	48.63pM
	0.3928	0
	0.0616	100nM
	0.0646	50nM
	0.0995	25nM
	0.1257	12.5nM
	0.1807	6.25nM
	0.2415	3.13nM
	0.2903	1.56nM
	0.3300	781.25pM
	0.3533	390.63pM
	0.3650	195.31pM
	0.3636	97.66pM
	0.3682	48.83pM
	0.3758	0

Kd = 4.53nM
ABC = 62.92pM
Ratio = 0.0139
Sig 100% = 0.40
Drift = -0.0110
(%/run)
NSB = 0.04
Drift = -2.0021
(mV/run)
%Error = 1.53



Kd = 4.53nM
95% confidence interval
Kd High = 5.09nM
Kd Low = 3.33nM



ABC = 62.92pM
95% confidence interval
ABC High = 2.59nM
ABC Low = Less than 227.30fM

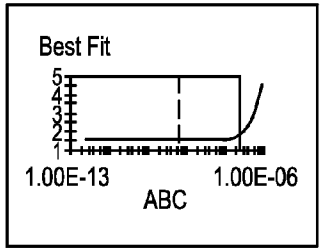
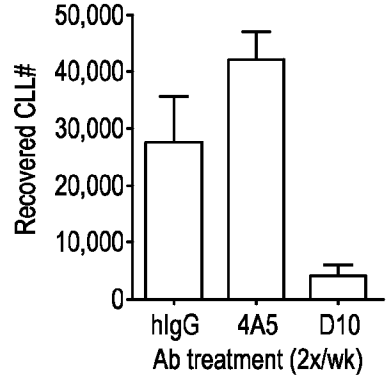
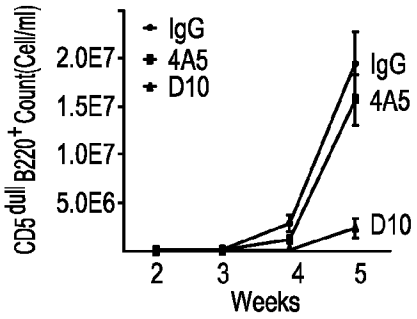


FIG. 16B

3-D10 Anti-R0R1 mAb is Highly Active in *in vivo* Assays

- 3-D10 Mab highly active in *in vivo* niche dependent activity model
  - Substantial reduction in leukemic burden using 4 primary CLL patient products tested in 76 mice
  - Activity much greater than other anti-R0R1 Mabs (4A5)
- 3-D10 Mab active in *in vivo* immune competent mouse model
  - Substantial reduction in spontaneous human R0R1 expressing leukemia model
  - Activity much greater than other anti-R0R1 Mabs (4A5)





3-D10 Mab has greatest anti-R0R1 activity in *in vivo* assay systems

FIG. 17

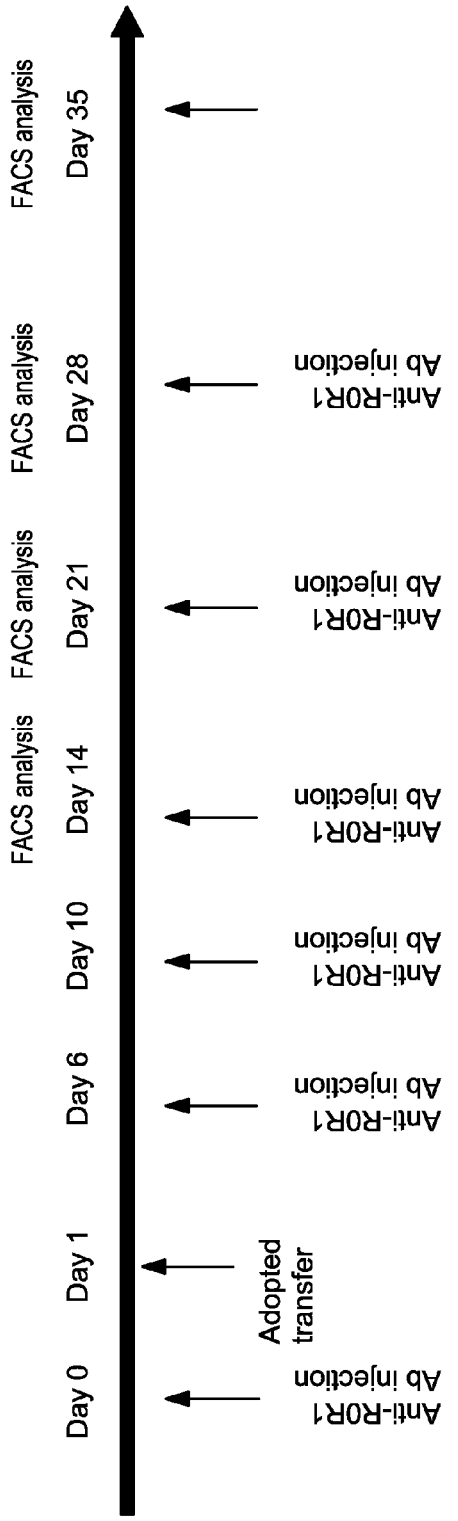

Day 0
Day 1
Day 6
Day 10
Day 14
Day 21
Day 28
Day 35
Anti-R0R1 Ab injection
Adopted transfer
Anti-R0R1 Ab injection
Anti-R0R1 Ab injection
Anti-R0R1 Ab injection
Anti-R0R1 Ab injection
Anti-R0R1 Ab injection
FACS analysis
FACS analysis
FACS analysis
FACS analysis

FIG. 18

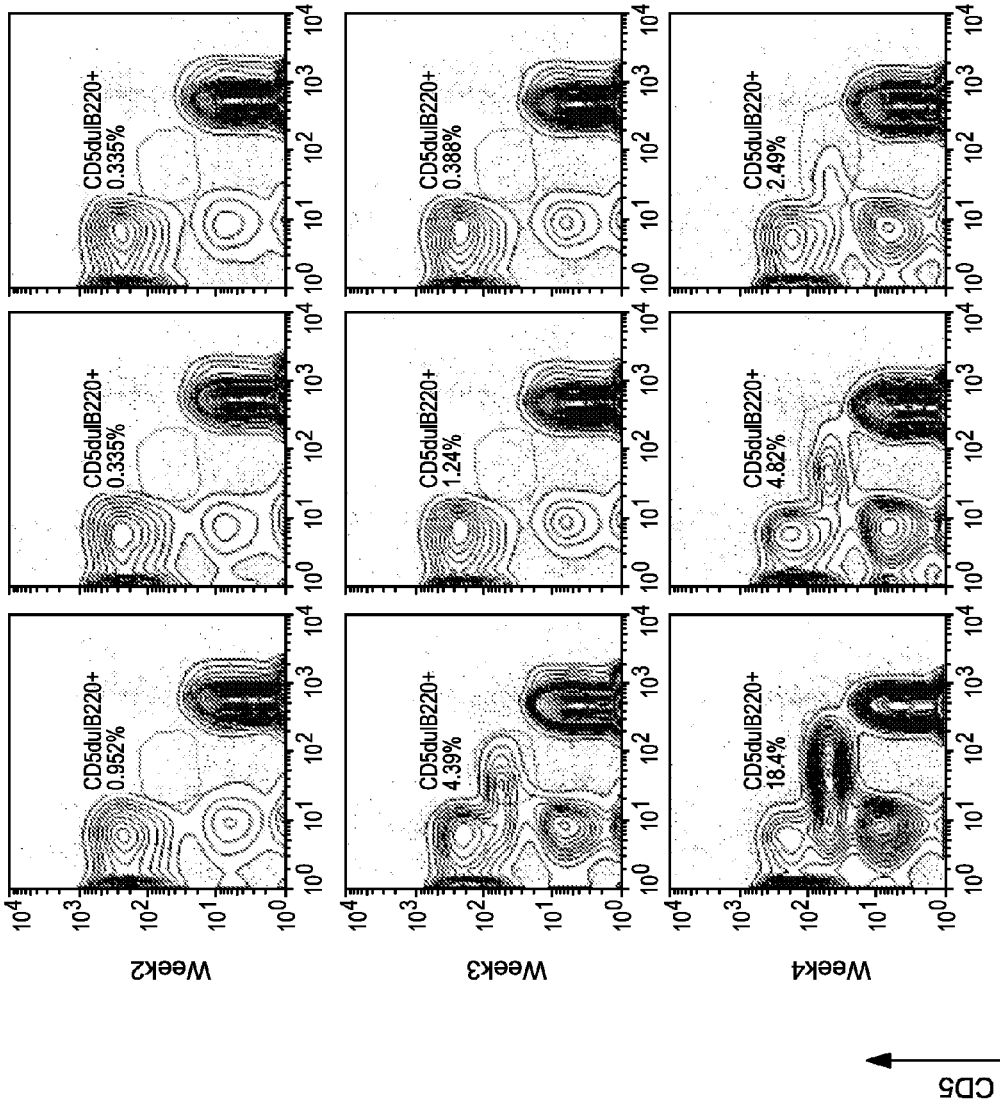

Week2
Week3
Week4
CD5dullB220+
0.952%
CD5dullB220+
0.335%
CD5dullB220+
0.335%
CD5dullB220+
4.39%
CD5dullB220+
1.24%
CD5dullB220+
0.388%
CD5dullB220+
18.4%
CD5dullB220+
4.82%
CD5dullB220+
2.49%
CD5
B220

FIG. 19

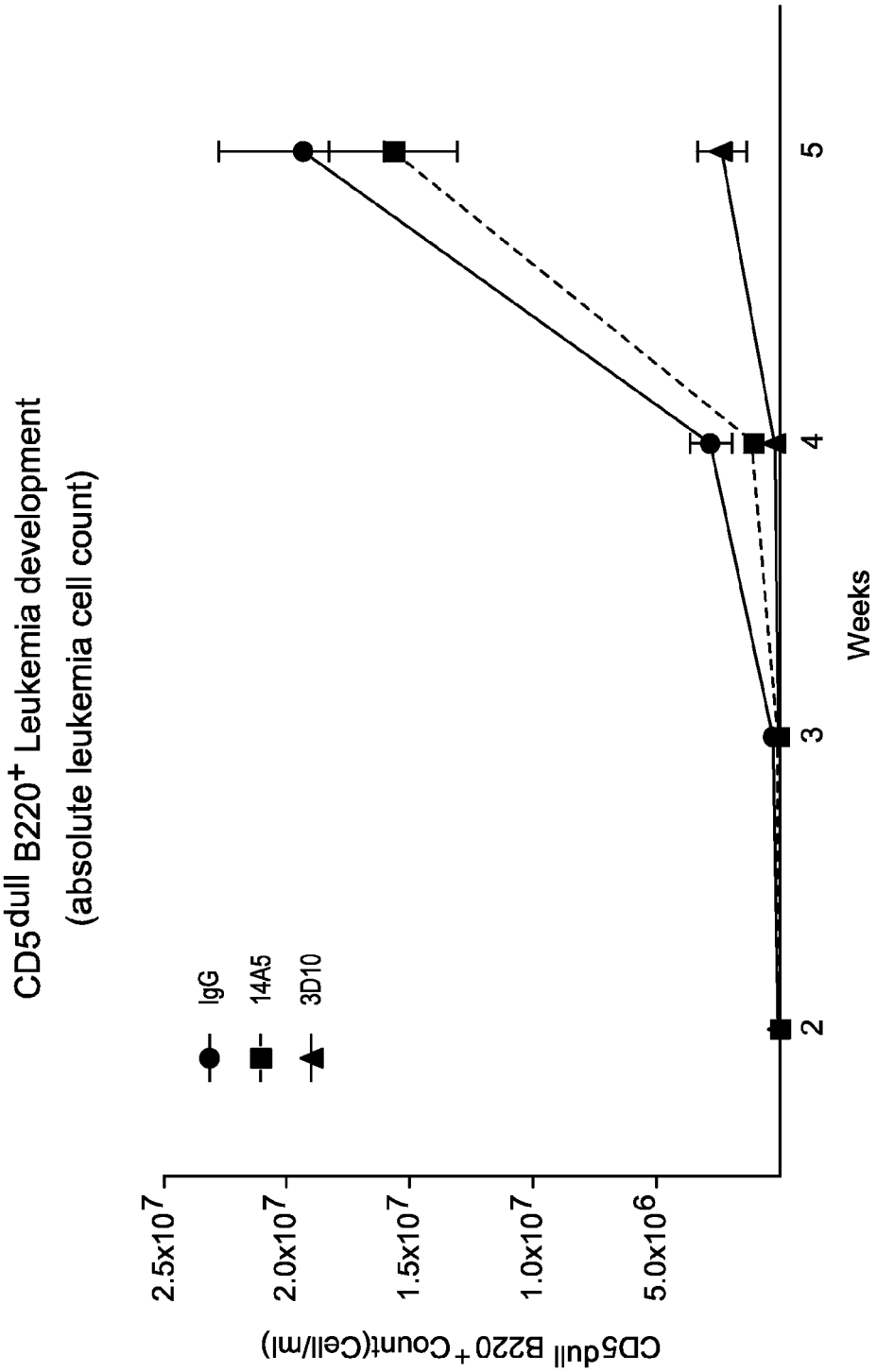

CD5dull B220+ Leukemia development
(absolute leukemia cell count)
IgG
14A5
3D10
2.5x10^7
2.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
CD5dull B220+ Count(Cell/ml)
2
3
4
5
Weeks

FIG. 20

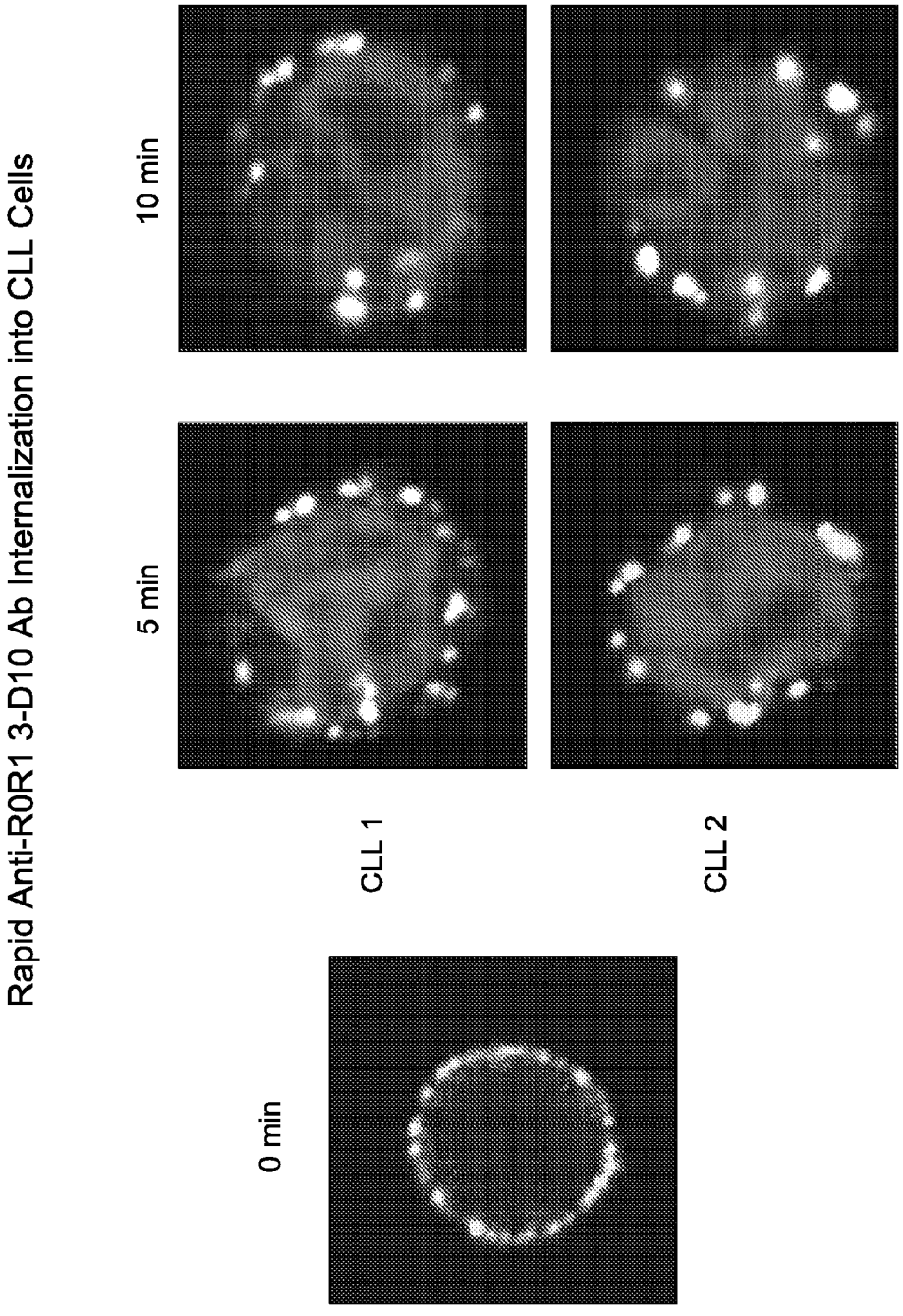

Rapid Anti-R0R1 3-D10 Ab Internalization into CLL Cells
10 min
5 min
0 min
CLL 1
CLL 2

FIG. 21

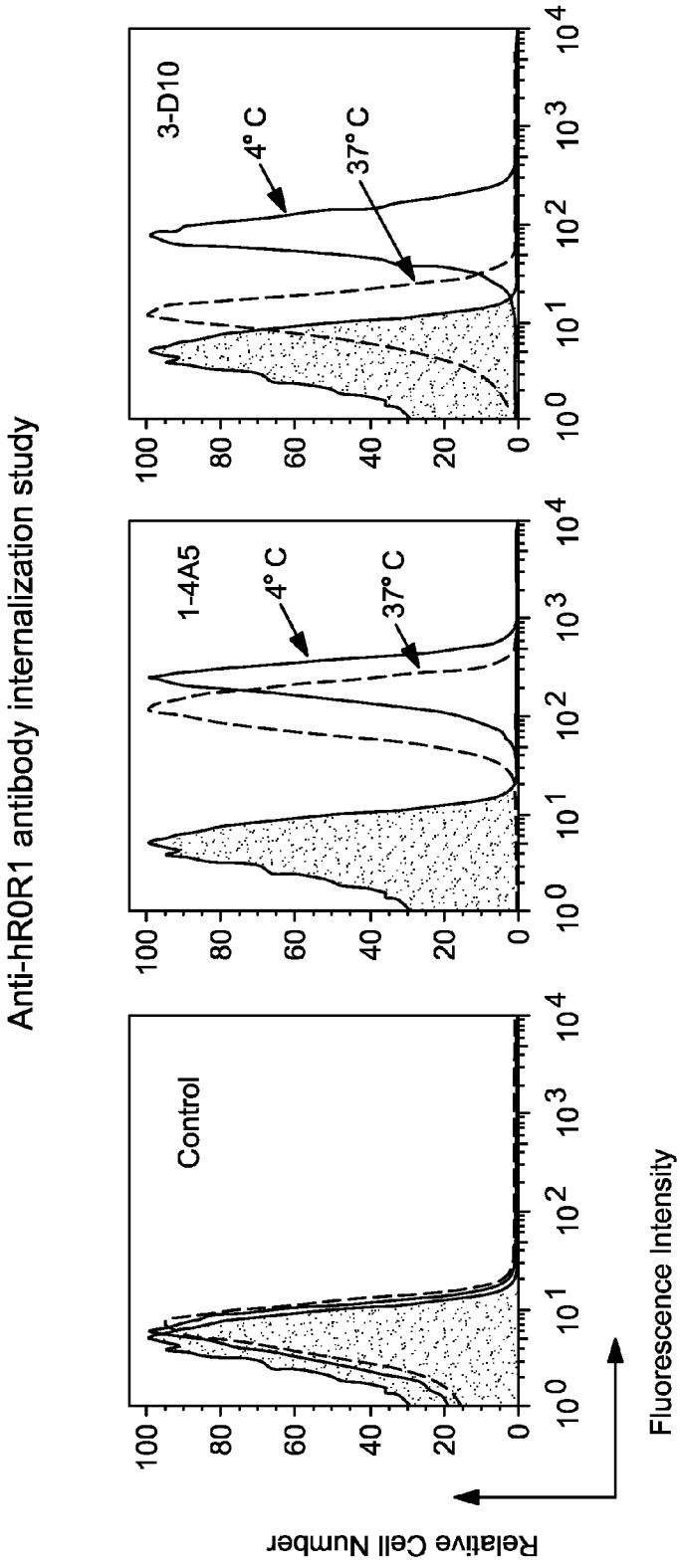

Anti-hR0R1 antibody internalization study
Control
1-4A5
3-D10
4° C
37° C
Relative Cell Number
Fluorescence Intensity

FIG. 22

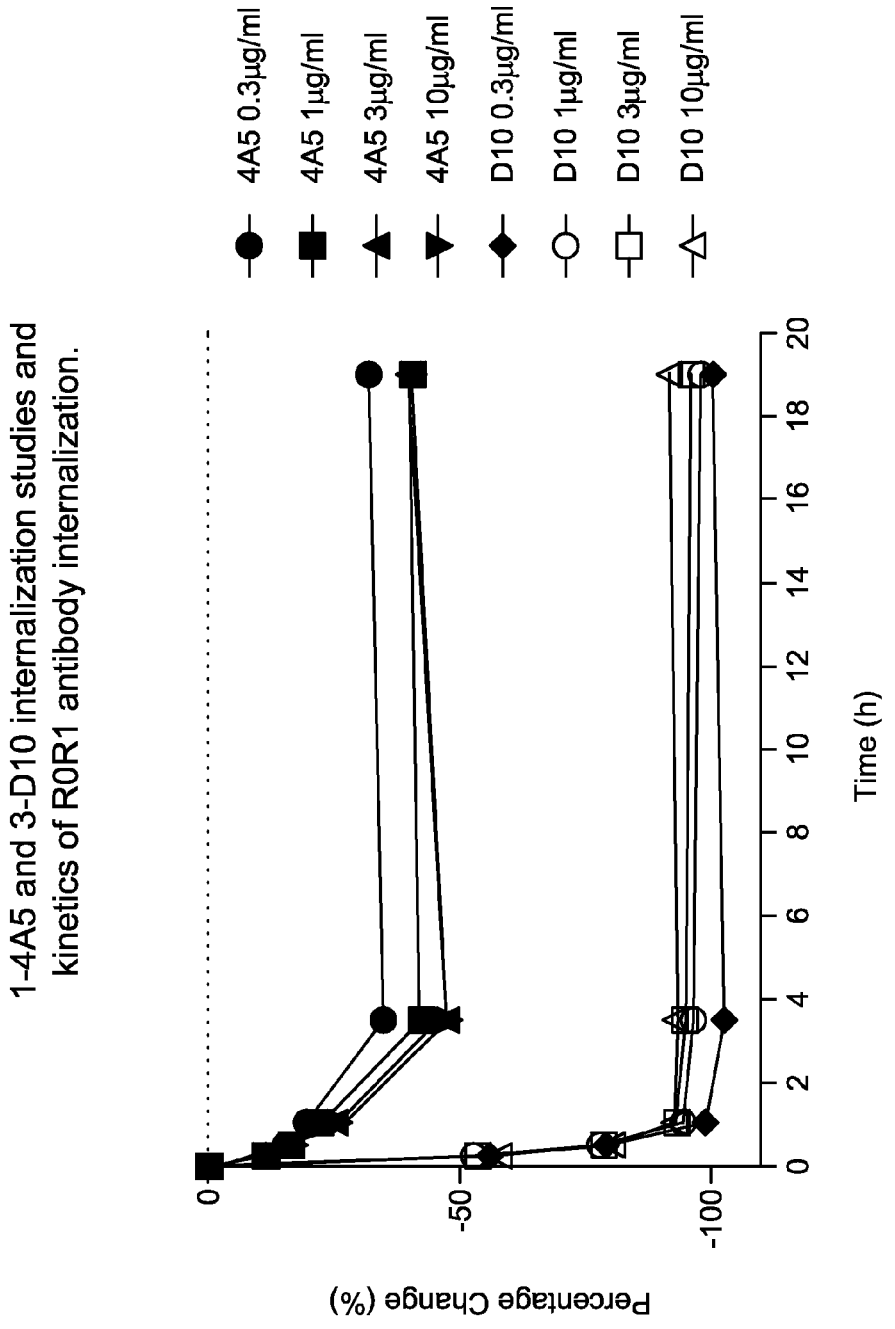

1-4A5 and 3-D10 internalization studies and kinetics of R0R1 antibody internalization.
4A5 0.3µg/ml
4A5 1µg/ml
4A5 3µg/ml
4A5 10µg/ml
D10 0.3µg/ml
D10 1µg/ml
D10 3µg/ml
D10 10µg/ml
Percentage Change (%)
0
-50
-100
0
2
4
6
8
10
12
14
16
18
20
Time (h)

FIG. 23

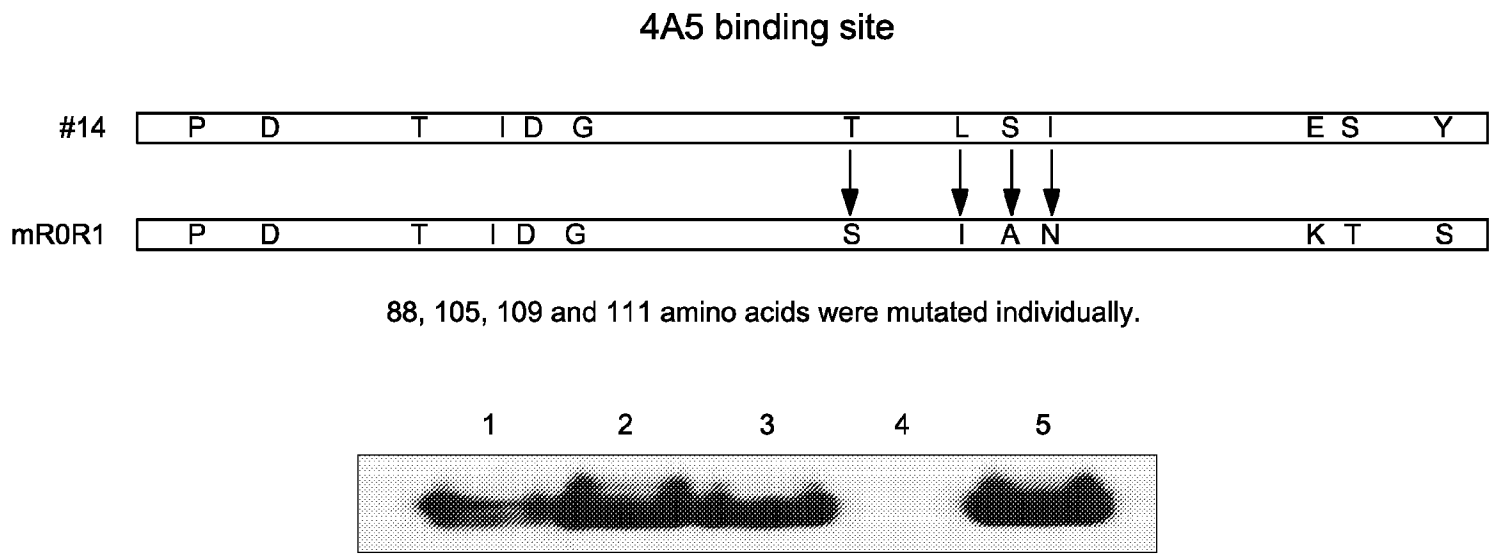

4A5 binding site
#14
P D T I D G T L S I E S Y
mR0R1
P D T I D G S I A N K T S
88, 105, 109 and 111 amino acids were mutated individually.
1 2 3 4 5
1. 88aa mutation
2. 105aa mutation
3. 109aa mutation
4. 111aa mutation
5. #14 protein (wild type)
4A5 binds to the 111 amino acid of human R0R1

FIG. 24

THERAPEUTIC ANTIBODIES AGAINST ROR-1 PROTEIN AND METHODS FOR USE OF SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 35 USC §371 National Stage application of International Application No. PCT/US2012/021339 filed Jan. 13, 2012, which claims the benefit under 35 USC §119(e) to U.S. Application Ser. No. 61/433,043 filed Jan. 14, 2011, now expired. The disclosure of each of the prior applications is considered part of and is incorporated by reference in the disclosure of this application.

GRANT INFORMATION

This invention was made with government support under Grant No. CA081534 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Tyrosine kinases are important mediators of the signaling cascade, determining key roles in diverse biological processes like growth, differentiation, metabolism and apoptosis in response to external and internal stimuli. Studies have implicated the role of tyrosine kinases in the pathophysiology of cancer. Schlessinger J. (2000) Cell, 103:211-225; and Robinson et al. (2000) Oncogene, 19:5548-5557. MacKeigan and colleagues used a large-scale RNAi approach to identify kinases that might regulate survival and apoptosis of a human tumor cell line (HeLa), RNAi to ROR1 was found as one of the most potent in inducing apoptosis among the set of RNAi targeting each of 73 different kinase-encoding genes. MacKeigan et al. (2005) Nat Cell Biol., 7:591-600. However, these investigators did not examine the expression or function of ROR1 protein in these cells.

ROR1, receptor tyrosine kinase like orphan receptor one, is a molecule expressed at high levels during embryogenesis that plays a major role in the development of the skeleton, lungs and nervous system. ROR1 expression is greatly decreased in postpartum mammalian cells to levels that are barely detectable. ROR1 is a membrane-receptor with an intracellular kinase-like domain and extracellular Frizzled-like cysteine-rich domain, which is common to receptors of members of the Wnt-family. ROR1 is member of the ROR family that is evolutionarily conserved among *Caenorhavditis elegans, Drosophila*, mice and humans. Wilson C, Goberdhan D C, Steller H. Dror, a potential neurotrophic receptor gene, encodes a Drosophila homolog of the vertebrate Ror family of Trk-related receptor tyrosine kinases. Proc Natl Acad Sci USA. 1993; 90:7109-7113; Oishi et al. (1997) J Biol Chem., 272:11916-11923; Masiakowski et al. (1992) J Biol Chem., 267:26181-26190; Forrester et al. (2002) Cell Mol Life Sci., 59:83-96; and Oishi et al. (1999) Genes Cells, 4:41-56. The actual functional role of the ROR1 protein during embryogenesis is unknown, although it is believed to be a receptor for Wnt proteins that regulate cellular polarity and cell-to-cell interactions.

Although principally an embryonic protein, ROR1 is expressed uniquely on certain cancer cells, including in CLL, small lymphocytic lymphoma, marginal cell B-Cell lymphoma, Burkett's Lymphoma, and other cancers (e.g., breast cancers), but not on normal adult tissues and cells. In a recent study, it was found that ROR1, at both mRNA and protein level, was highly expressed in CLL B cells but not normal B cells. Moreover, it was found that ROR1 is a receptor for Wnt5a, which could induce activation of NF-κB when co-expressed with ROR1 in HEK293 cells and enhance survival of CLL cells in vitro. This indicates that ROR1 is a CLL survival-signaling receptor for Wnt5a. Another study found that ROR1 was expressed in acute lymphocytic leukemia (ALL) as well. Shabani et al. (2007) Tumour Biol., 28:318-326; and Baskar et al. (2008) Clin Cancer Res., 14:396-404. Expression of ROR1 protein has now been demonstrated on a variety of hematologic and solid tumor cancers.

Therapeutic control of ROR1 expression is necessary. However, although polyclonal anti-ROR1 antibodies raised against ROR1 peptide are commercially available. The inventors developed a monoclonal anti-ROR1 antibody, terms 4A5, which reacts with the native ROR1 protein and is capable of detecting cell-surface expression of ROR1 for flow cytometric analysis. However, robustly therapeutic antibodies with demonstrable ability to inhibit ROR-1 mediated cancer cell proliferation to a degree that is therapeutically significant for slowing or preventing growth and metastasis have not been available.

SUMMARY OF THE INVENTION

The invention provides antibodies and combination of antibodies for in vivo and in vitro inhibition of ROR-1 cell mediated proliferation of cells from subjects with cancer, including lymphomas, CLL, small lymphocytic lymphoma, marginal cell B-Cell lymphoma, Burkett's Lymphoma, renal cell carcinoma, colon cancer, colorectal cancer, breast cancer, epithelial squamous cell cancer, melanoma, myeloma, stomach cancer, brain cancer, lung cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, prostate cancer, testicular cancer, thyroid cancer, and head and neck cancer, but not in blood or splenic lymphocytes of nonleukemic patients or normal adults.

The antibodies of the invention are also useful for differentiation between ROR1 expressing cancer cells ("ROR1 cancer") and normal cells. For example, an immunoassay that detects ROR1 in a sample from a subject by contacting the sample with a ROR1-specific antibody of the invention and detecting immunoreactivity between the antibody and ROR1 in the sample is provided.

In accordance with a further aspect of the invention, a ROR1 cancer is diagnosed in a subject by detecting the presence or quantity of ROR1 protein in a sample.

The present invention includes compositions that include purified, isolated monoclonal antibodies and combinations thereof that bind specifically to ROR1 receptor protein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a series of graphs illustrating the results of flow cytometric analysis of the expansion of CD5+B220low leukemia B cells in ROR1 Tg mice following the adoptive transfer of 1×10^7 splenocytes from a ROR1 xTCL1 Tg mouse. Upper panel depicts the expansion from 2 to 4 weeks following adoptive transfer. Percentage of leukemic cells on the contour plot of mCD5 (x-axis) vs mB220 (y-axis) is indicated on above the gate on $CD5^{+}B220^{low}$ lymphocytes. Bottom panel depicts the relative ROR1 expression (x axis) using the mouse anti-ROR1 4A5 mAb.

FIG. **2** is a diagram outlining the analysis of anti-ROR1 mAb on the adoptive transfer and engragment of ROR1 XTCL1 leukemic splenocytes. ROR1 Tg mice (4 mice/group) were given 250 ug of 4A5, D10 or control mIgG i.v. on day 0. The following day, 1×10^7 splenocytes from a ROR1 x TCL1

Tg mouse were adoptively transferred i.v. All mice were subsequently monitor weekly for expansion of $CD5^{+}B220^{low}$ leukemic B cells by flow cytometry beginning at 2 weeks post transfer.

FIG. **3** is a series of graphs illustrating the results of a flow cytometric analysis which demonstrate that anti-ROR1 antibodies of the invention inhibited the development of CLL-like leukemia in ROR1 Tg mice. 2 weeks after adoptive transfer, the PBMC facs analysis were performed. The data showed the anti-ROR1 antibody D10 but not anti-ROR1 antibody 4A5 could markedly inhibit the $CD5^{dull}B220^{+}$ and $ROR1^{bright}B220^{+}$ leukemic B cell expansion.

FIG. **4**A is a series of graphs illustrating the results of in vivo testing in a murine model of human breast cancer. The anti-ROR1 antibodies inhibited breast cancer metastasis in rag−/−g−/− deficiency mice. 5E5 MDA-MB-231 breast cancer cell were transferred by i.v. injection to rag−/−g−/− mice on day 1. The rag−/−g−/− deficiency mice were also i.v. injected isotype control or anti-ROR1 antibody (4A5, D10, and 4A5 plus D10) on day 1, 3, 7 and 14 at 100 mg per mice. FIG. **4**A (center) also provides images from IVIS in vivo imaging procedures on the above mice, which were performed every week. 5 weeks later, the mice were sacrificed and histology analysis were performed (FIG. **4**B). The anti-ROR1 antibody D10 and the antibody combination (4A5 plus D10) both significantly inhibited metastasis of the breast cancer, with inhibition by D10 alone being greater than inhibition by 4a5 alone.

FIG. **5** provides a nucleotide coding sequence comparison of 4A5 Ig heavy chain (VH) to the closest germline mouse and human immunoglobulin (Ig) VH.

FIG. **6** provides a nucleotide coding sequence comparison of G6 Ig heavy chain (VH) to the closest germline mouse and human immunoglobulin (Ig) VH.

FIG. **7** provides a nucleotide coding sequence comparison of G3 Ig heavy chain (VH) to the closest germline mouse and human immunoglobulin (Ig) VH.

FIG. **8** provides a nucleotide coding sequence comparison of H10 Ig heavy chain (VH) to the closest germline mouse and human immunoglobulin (Ig) VH.

FIG. **9** provides a nucleotide coding sequence comparison of D10 Ig heavy chain (VH) to the closest germline mouse and human immunoglobulin (Ig) VH.

FIG. **10** is a diagram and chart depicting the highly conserved nature of human and murine ROR1.

FIG. **11** is a nucleotide comparison depicting the domain structure and sequence homology of human and murine ROR1 extracellular protein.

FIG. **12** is a chart indicating the extracellular domain which the anti-ROR1 mAbs bind the ROR1 protein.

FIG. **13** is a diagram depicting the chimeric ROR1 proteins generated to determine the binding domain of each of the anti-ROR1 mAbs.

FIG. **14** is a diagram depicting the truncated ROR1 proteins generated to determine the sub-regions which each of the anti-ROR1 mAbs binds.

FIG. **15** is a diagram depicting the amino acids which were murinized to determine residues critical for mAb binding to human ROR1 and a western blot showing that the 138 glutamic acid residue is critical for antibody D10 binding to human ROR1.

FIG. **16** is a graph indicating the K_D values for antibody D10 (FIG. **16***a*) and 4A5 (FIG. **16***b*).

FIG. **17** is a series of graphs illustrating the anti-ROR1 antibody D10 is highly active in in vivo assays.

FIG. **18** is a diagram outlining the analysis of anti-ROR1 mAb on the adoptive transfer and engragment of ROR1XTCL1 leukemic splenocytes. ROR1 Tg mice (5 mice/group) were given 250 ug of 4A5, D10 or control mIgG i.v. on day 0. The following day, 5×10^{5} splenocytes from a ROR1 X TCL1 Tg mouse were adoptively transferred i.v. All mice were subsequently monitored weekly for expansion of $CD5^{dull}B200^{+}$ leukemic B cells by flow cytometery beginning at 2 weeks post transfer.

FIG. **19** a series of graphs illustrating the results of flow cytometric analysis of the anti-ROR1 antibodies inhibiting the development of CLL-like leukemia in ROR1 Tg mice. 2 weeks after adoptive transfer, the PBMC facs analysis were performed. The data showed the anti-ROR1 antibody D10 but not anti-ROR1 antibody 4A5 could markedly inhibit the $CD5^{dull}B220^{+}$ and $ROR1^{bright}B220^{+}$ leukemic B cell expansion.

FIG. **20** is a graph illustrating that anti-ROR1 antibody D10 inhibits the development and expansion of ROR1xTCL1 leukemic B cells in the blood of recipient animals until two weeks after receiving the last infusion of the mAb.

FIG. **21** is a depiction of the rapid internalization of the anti-ROR1 antibody D10 into CLL cells.

FIG. **22** is a series of graphs illustrating the results of flow cytometric analysis showing that anti-ROR1 antibodies D10 and 4A5 are both internalized into CLL cells. CLL cells were incubated with mouse anti-hROR1 Ab-Alex647 for 30 min at 4° C. Subsequently the cells were washed and either left at 4° C. or incubated for 4 hours at 37° C., followed by flow cytometry. The background signal with non-staining is also shown.

FIG. **23** is a graph illustrating the kinetics of the internalization of anti-ROR1 antibodies D10 and 4A5.

FIG. **24** is a diagram depicting the amino acids which were murinized to determine residues critical for mAb binding to human ROR1 and a western blot showing that the 111 isoleucine residue is critical for antibody 4A5 binding to human ROR1.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The presently disclosed subject matter are described more fully below. However, the presently disclosed subject matter may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Indeed, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions and the associated Figures. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims.

Antibodies of the invention were produced monoclonally using techniques as previously described. Briefly, Naturally occurring antibodies are generally tetramers containing two light chains and two heavy chains. Experimentally, antibodies can be cleaved with the proteolytic enzyme papain, which causes each of the heavy chains to break, producing three separate subunits. The two units that consist of a light chain and a fragment of the heavy chain approximately equal in mass to the light chain are called the Fab fragments (i.e., the antigen binding fragments). The third unit, consisting of two equal segments of the heavy chain, is called the Fc fragment.

The Fc fragment is typically not involved in antigen-antibody binding, but is important in later processes involved in ridding the body of the antigen.

Because Fab and $F(ab')_2$ fragments are smaller than intact antibody molecules, more antigen-binding domains are available than when whole antibody molecules are used. Proteolytic cleavage of a typical IgG molecule with papain is known to produce two separate antigen binding fragments called Fab fragments which contain an intact light chain linked to an amino terminal portion of the contiguous heavy chain via by disulfide linkage. The remaining portion of the papain-digested immunoglobin molecule is known as the Fc fragment and consists of the carboxy terminal portions of the antibody left intact and linked together via disulfide bonds. If an antibody is digested with pepsin, a fragment known as an $F(ab')_2$ fragment is produced which lacks the Fc region but contains both antigen-binding domains held together by disulfide bonds between contiguous light and heavy chains (as Fab fragments) and also disulfide linkages between the remaining portions of the contiguous heavy chains (Handbook of Experimental Immunology. Vol 1: Immunochemistry, Weir, D. M., Editor, Blackwell Scientific Publications, Oxford (1986)).

As readily recognized by those of skill in the art, altered antibodies (e.g., chimeric, humanized, CDR-grafted, bifunctional, antibody polypeptide dimers (i.e., an association of two polypeptide chain components of an antibody, e.g., one arm of an antibody including a heavy chain and a light chain, or an Fab fragment including VL, VH, CL and CH antibody domains, or an Fv fragment comprising a VL domain and a VH domain), single chain antibodies (e.g., an scFv (i.e., single chain Fv) fragment including a VL domain linked to a VH domain by a linker, and the like) can also be produced by methods well known in the art.

Monoclonal antibody (mAb) technology can be used to obtain mAbs to ROR1. Briefly, hybridomas are produced using spleen cells from mice immunized with ROR1 antigens. The spleen cells of each immunized mouse are fused with mouse myeloma Sp 2/0 cells, for example using the polyethylene glycol fusion method of Galfre, G. and Milstein, C., Methods Enzymol., 73:3-46 (1981). Growth of hybridomas, selection in HAT medium, cloning and screening of clones against antigens are carried out using standard methodology (Galfre, G. and Milstein, C., Methods Enzymol., 73:3-46 (1981)):

HAT-selected clones are injected into mice to produce large quantities of mAb in ascites as described by Galfre, G. and Milstein, C., Methods Enzymol., 73:3-46 (1981), which can be purified using protein A column chromatography (Bio-Rad, Hercules, Calif.). mAbs are selected on the basis of their (a) specificity for ROR1, (b) high binding affinity, (c) isotype, and (d) stability.

mAbs can be screened or tested for ROR1 specificity using any of a variety of standard techniques, including Western Blotting (Koren, E. et al., Biochim. Biophys. Acta 876:91-100 (1986)) and enzyme-linked immunosorbent assay (ELISA) (Koren, E. et al., Biochim. Biophys. Acta 876:91-100 (1986)).

Humanized forms of mouse antibodies can be generated by linking the CDR regions of non-human antibodies to human constant regions by recombinant DNA techniques (see, e.g., Queen et al., Proc. Natl. Acad. Sci. USA 86:10029-10033, 1989 and WO 90/07861, each incorporated by reference). Human antibodies can be obtained using phage-display methods (see, e.g., Dower et al., WO 91/17271; McCafferty et al., WO 92/01047). In these methods, libraries of phage are produced in which members display different antibodies on their outer surfaces. Antibodies are usually displayed as Fv or Fab fragments. Phage displaying antibodies with a desired specificity may be selected by affinity enrichment.

Human antibodies may be selected by competitive binding experiments, or otherwise, to have the same epitope specificity as a particular mouse antibody. Using these techniques, a humanized ROR1 antibody having the human IgG1 constant region domain and the human kappa light chain constant region domain with the mouse heavy and light chain variable regions. The humanized antibody has the binding specificity of a mouse ROR1 mAb, specifically the 4A5 mAb described in Examples 4 and 5.

It may be desirable to produce and use functional fragments of a mAb for a particular application. The well-known basic structure of a typical IgG molecule is a symmetrical tetrameric Y-shaped molecule of approximately 150,000 to 200,000 daltons consisting of two identical light polypeptide chains (containing about 220 amino acids) and two identical heavy polypeptide chains (containing about 440 amino acids). Heavy chains are linked to one another through at least one disulfide bond. Each light chain is linked to a contiguous heavy chain by a disulfide linkage. An antigen-binding site or domain is located in each arm of the Y-shaped antibody molecule and is formed between the amino terminal regions of each pair of disulfide linked light and heavy chains. These amino terminal regions of the light and heavy chains consist of approximately their first 110 amino terminal amino acids and are known as the variable regions of the light and heavy chains. In addition, within the variable regions of the light and heavy chains there are hypervariable regions which contain stretches of amino acid sequences, known as complementarity determining regions (CDRs). CDRs are responsible for the antibody's specificity for one particular site on an antigen molecule called an epitope. Thus, the typical IgG molecule is divalent in that it can bind two antigen molecules because each antigen-binding site is able to bind the specific epitope of each antigen molecule. The carboxy terminal regions of light and heavy chains are similar or identical to those of other antibody molecules and are called constant regions. The amino acid sequence of the constant region of the heavy chains of a particular antibody defines what class of antibody it is, for example, IgG, IgD, IgE, IgA or IgM. Some classes of antibodies contain two or more identical antibodies associated with each other in multivalent antigen-binding arrangements.

Fab and $F(ab')_2$ fragments of mAbs that bind ROR1 can be used in place of whole mAbs. Because Fab and $F(ab')_2$ fragments are smaller than intact antibody molecules, more antigen-binding domains are available than when whole antibody molecules are used. Proteolytic cleavage of a typical IgG molecule with papain is known to produce two separate antigen binding fragments called Fab fragments which contain an intact light chain linked to an amino terminal portion of the contiguous heavy chain via by disulfide linkage. The remaining portion of the papain-digested immunoglobin molecule is known as the Fc fragment and consists of the carboxy terminal portions of the antibody left intact and linked together via disulfide bonds. If an antibody is digested with pepsin, a fragment known as an $F(ab')_2$ fragment is produced which lacks the Fc region but contains both antigen-binding domains held together by disulfide bonds between contiguous light and heavy chains (as Fab fragments) and also disulfide linkages between the remaining portions of the contiguous heavy chains (Handbook of Experimental Immunology. Vol 1: Immunochemistry, Weir, D. M., Editor, Blackwell Scientific Publications, Oxford (1986)).

With respect to particular antibodies, "specific binding" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with an affinity corresponding to a K_D of about 10^{-8} M or less, and binds to the predetermined antigen with an affinity (as expressed by K_D) that is at least 10 fold less, and preferably at least 100 fold less than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. Alternatively, the antibody can bind with an affinity corresponding to a K_A of about 10^6 M^{-1}, or about 10^7 M^{-1}, or about $10^8 M^{-1}$, or 10^9 M^{-1} or higher, and binds to the predetermined antigen with an affinity (as expressed by K_A) that is at least 10 fold higher, and preferably at least 100 fold higher than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen.

Also, reference to "an antibody having binding specificity for ROR-1 protein" includes antibody fragments having at least 90% or 95% sequence identity to any of the polypeptide sequences disclosed in SEQ ID NOs: 2. 4 6, 8, 12, 14, 16, 18 and 20, including variants modified by mutation to improve the utility thereof (e.g., improved ability to target specific cell types and the like). Such variants include those wherein one or more conservative substitutions are introduced into the heavy chain and/or the light chain of the antibody.

Such variants include those wherein one or more substitutions are introduced into the heavy chain nucleotide sequence and/or the light chain nucleotide sequence of the antibody. In some embodiments the variant has a light chain and/or heavy chain having a nucleotide sequence at least 80% or at least 90% or at least 95% identical to any of the nucleotide sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 11, 13, 15, 17 and 19.

Polynucleotide sequences which code structural features of the antibodies of the invention include those whose sequences are set forth below. Each polynucleotide sequence is followed by the amino acid sequence of the encoded polypeptide. The light chain sequences which are considered to be "corresponding" to heavy chain sequences are those listed as being for the same antibody; i.e., the F2 heavy chain sequences correspond to the F2 light chain sequences, the D10 heavy chain sequences correspond to the D10 light chain sequences, and so forth.

SEQ ID NO: 1 4A5 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Coding Sequence:

```
GAAGTGAAACTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTC
CTGTGCAGCCTCTGGATT
CACTTTCAGTAGCTATGCCATGTCTTGGGTTCGCCAGATTCCAGAGAAGAGGCTGGAGTGGG
TCGCATCCATTAGTCGTG
GTGGTACCACCTACTATCCAGACAGTGTGAAGGGCCGATTCACCATCTCCAGAGATAATGTC
AGGAACATCCTGTACCTG
CAAATGAGCAGTCTGAGGTCTGAGGACACGGCCATGTATTACTGTGGAAGATATGATTACGA
CGGGTACTATGCAATGGA
CTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA
```

SEQ ID NO: 2 4A5 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Polypeptide Sequence:

```
EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQIPEKRLEWVASISRGGTTYYPDS
VKGRFTISRDNVRNILYL
QMSSLRSEDTAMYYCGRYDYDGYYAMDYWGQGTSVTVSS
```

SEQ ID NO: 3 4A5 Mouse Anti-ROR1 mAb Light Chain Variable Region Coding Sequence:

```
GACATCAAGATGACCCAGTCTCCATCTTCCATGTATGCATCTCTAGGAGAGAGAGTCACTAT
CACTTGCAAGGCGAGTCC
GGACATTAATAGCTATTTAAGCTGGTTCCAGCAGAAACCAGGGAAATCTCCTAAGACCCTGA
TCTATCGTGCAAACAGAT
TGGTTGATGGGGTCCCATCAAGGTTCAGTGGCGGTGGATCTGGGCAAGATTATTCTCTCACC
ATCAACAGCCTGGAGTAT
GAAGATATGGGAATTTATTATTGTCTACAGTATGATGAATTTCCGTACACGTTCGGAGGGGG
GACCAAGCTGGAAATGAA
AC
```

SEQ ID NO: 4 4A5 Mouse Anti-ROR1 mAb Light Chain Variable Region Polypeptide Sequence:

DIKMTQSPSSMYASLGERVTITCKASPDINSYLSWFQQKPGKSPKTLIYRANRLVDGVPSRF
SGGGSGQDYSLTINSLEY
EDMGIYYCLQYDEFPYTFGGGTKLEMK

SEQ ID NO: 5 F2, F12 and G6 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Coding Sequence:

GAGGTCCAGCTACAGCAGTCTGGACCTGAGCTGGAGAAGCCTGGCGCTTCAGTGAAGATATC
CTGCAAGGCTTCTGGTTT
CGCATTCACTGGCTACAACATGAACTGGGTGAAACAGACCAATGGAAAGAGCCTTGAGTGGA
TTGGAAGTATTGATCCTT
ACTATGGTGGTTCTACCTACAACCAGAAGTTCAAGGACAAGGCCACATTGACTGTAGACAAA
TCCTCCAGCACAGCCTAC
ATGCAACTCAAGAGCCTCACATCTGATGACTCTGCAGTCTATTACTGTGCAAGATCCCCGGG
GGGGGACTATGCTATGGA
CTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA

SEQ ID NO: 6 F2, F12 and G6 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Polypeptide Sequence:

EVQLQQSGPELEKPGASVKISCKASGFAFTGYNMNWVKQTNGKSLEWIGSIDPYYGGSTYNQ
KFKDKATLTVDKSSSTAY
MQLKSLTSDDSAVYYCARSPGGDYAMDYWGQGTSVTVSS

SEQ ID NO: 7 F2, F12 and G6 Mouse Anti-ROR1 mAb Light Chain Variable Region Coding Sequence:

GACATCAAGATGACCCAGTCTCCATCTTCCATGTATGCATCTGTAGGAGAGAGAGTCACTAT
CACTTGTAAGGCGAGTCA
GGGCATTAATAGCTATTCAGGCTGGTTCCAGCAGAAACCAGGGAAATCTCCTAAGACCCTGA
TTTATCGTGGAAATAGAT
TGGTGGATGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGCAAGATTATTCTCTCACC
ATCAGCAGCCTGGAGTAT
GAAGATATGGGAATTTATTATTGTCTACAGTATGATGAGTTTCCGTACACGTTCGGAGGGGG
GACCAAGCTGGAAATAAA
AC

SEQ ID NOs: 8 F2, F12 and G6 Mouse Anti-ROR1 mAb Light Chain Variable Region Polypeptide Sequence:

DIKMTQSPSSMYASVGERVTITCKASQGINSYSGWFQQKPGKSPKTLIYRGNRLVDGVPSRF
SGSGSGQDYSLTISSLEY
EDMGIYYCLQYDEFPYTFGGGTKLEIK

SEQ ID NO: 9 G3 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Coding Sequence:

CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTTGTGAAGCCTGGGACTTCAGTGAAGCTGTC

CTGCAAGGCTTCTGGCTA

CAACTTCACCAACTACTGGATAAACTGGGTGAAGCTGAGGCCTGGACAAGGCCTTGAGTGGA

TTGGAGAAATTTATCCTG

GTAGTGGTAGTACTAATTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGCAGACACA

TCCTCCAGCACAGCCTAC

ATGCAACTCAGCAGCCTGGCATCTGAAGACTCTGCTCTCTATTACTGTGCAAGAGATGGTAA

CTACTATGCTATGGACTA

CTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA

SEQ ID NO: 10 G3 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Polypeptide Sequence:

QVQLQQPGAELVKPGTSVKLSCKASGYNFTNYWINWVKLRPGQGLEWIGEIYPGSGSTNYNE

KFKSKATLTADTSSSTAY

MQLSSLASEDSALYYCARDGNYYAMDYWGQGTSVTVSS

SEQ ID NO: 11 G3 Mouse Anti-ROR1 mAb Light Chain Variable Region Coding Sequence:

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TCTACTACACATCAGCAT

TACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACC

ATTAGCAACCTGGAACAA

GAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCTCCGTACACGTTCGGAGG

GGGGACCAAGCTGGAAAT

AAAAC

SEQ ID NO: 12 G3 Mouse Anti-ROR1 mAb Light Chain Variable Region Polypeptide Sequence:

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SEQ ID NO: 14 D10 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Polypeptide Sequence

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ATGAAATATCCAAACTGG

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AGCAGCATGGAGGCTGAA

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SEQ ID NO: 16 D10 Mouse Anti-ROR1 mAb Light Chain Variable Region Polypeptide Sequence:

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SEQ ID NO: 17 H10 and G11 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Coding Sequence:

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GTGCTAGCGCCTACTTTCCAGACAGTGTGAAGGGCCGATTCACCATCTCCAGAGATAATGCC

AGGAACATCCTGTACCTG

CAAATGAGCAGTCTGAGGTCTGAGGACACGGCCATGTATTATTGTGCAAGGATTACTACGTC

TACCTGGTACTTCGATGT

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SEQ ID NO: 18 H10 and G11 Mouse Anti-ROR1 mAb Heavy Chain Variable Region Polypeptide Sequence:

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VKGRFTISRDNARNILYL

QMSSLRSEDTAMYYCARITTSTWYFDVWGAGTTVTVSS

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TCTATCGTGCAAACAGAT

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ATCAGCAGCCTGGAGTAT

GAAGATATGGGAATTTATTATTGTCTACAGTATGATGAGTTTCCGTACACGTTCGGAGGGGG

GACCAAGCTGGAAATAAA

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SEQ ID NO: 20 H10 and G11 Mouse Anti-ROR1 mAb Light Chain Variable Region Polypeptide Sequence:

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SGSGSGQDYSLTISSLEY

EDMGIYYCLQYDEFPYTFGGGTKLEIK

In one aspect, antibodies are provided in which a heavy chain encoded by the polynucleotide sequence of SEQ ID NO:13 and a light chain encoded by the polynucleotide sequence of SEQ ID NO:15.

In another aspect, an antibody of the present invention contains a heavy chain encoded by the polynucleotide sequence of SEQ ID NO:1 and a light chain encoded by the polynucleotide sequence of SEQ ID NO:3.

In further aspects, antibodies are provided which have a heavy chain encoded by the polynucleotide sequence of SEQ ID NO: 5 and a light chain encoded by the polynucleotide sequence of SEQ ID NO: 7; or by the polynucleotide sequence of SEQ ID NO: 9 and alight chain encoded by the polynucleotide sequence of SEQ ID NO: 11; or by the polynucleotide sequence of SEQ ID NO: 15 and a light chain encoded by the polynucleotide sequence of SEQ ID NO: 17.

In another aspect, antibodies are provided which contain a heavy chain with the polypepetide sequence of SEQ ID NO:14 and a light chain with the polypeptide sequence of SEQ ID NO:16.

In another aspect, antibodies are provided which contain a heavy chain with the polypeptide sequence of SEQ ID NO:2 and a light chain with the polypeptide sequence of SEQ ID NO:4.

In one embodiment, isolated polynucleotides which encode an antibody that specifically binds ROR1 protein are provided which are (a) comprised of a heavy chain region coded by polynucleotides having at least 90% sequence identity with any of the sequences selected from the group consisting of SEQ ID NOs: 1, 5, 9, 13 or 17, (b) comprised of a corresponding light chain region encoded by polynucleotides having at least 90% sequence identity with any of the sequences selected from the group consisting of SEQ ID NOs: 3, 7, 11, 15 or 19, and (c) specifically binds either the 3' end or middle portion of the Ig-like region of the extracellular domain of human or murine ROR-1 protein.

Also provided are antibodies which bind residues within the middle of the Ig-like region of the extracellular domain of human or murine ROR-1 protein (amino acids 1-147 in the human molecule). In one aspect, the antibodies of the present invention bind to amino acids 70-130 of human ROR1. Examples of such antibodies include 4A5, G11, H10 and G3.

Alternatively or additionally, a residue corresponding to the one found in the extracellular domain of human ROR-1 protein at position 111 is critical to the binding activity of the antibodies.

Further provided are antibodies that bind residues within the 3' Ig-like region and the linker region between the Ig-like domain and the CRD domain of human or murine ROR-1 protein (amino acids 1-165 in the human molecule). In one aspect, the antibodies of the present invention bind to amino acids 130-165 of human ROR1. Examples of such antibodies include D10, F2, F12 and G6.

Alternatively or additionally, the antibodies bind a glutamic acid residue corresponding to the one found in the extracellular domain of human ROR-1 protein at position 138.

Alternatively or additionally, a residue corresponding to the one found in the extracellular domain of human ROR-1 protein at position 138 is critical to the binding activity of the antibodies.

Alternatively or additionally, the encoded antibody has in vivo activity in reducing leukemic or lymphomic cell burden in an art-accepted animal model at a rate of 2-8 times, or at least 2, 3, 4, 5, 6, 7, or 8 times, that of wild-type human anti-ROR1 antibody or monoclonal 4A5 antibody (disclosed herein).

Alternatively or additionally, the encoded antibody has in vivo activity in inhibiting $CD5^{dull}B220^{+}$ and $ROR1^{bright}B220^{+}$ leukemic B cell expansion.

Alternatively or additionally, the encoded antibody is internalized into leukemic or lymphomic cells at a rate of at least 2 times, or at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 times that of monoclonal antibody 4A5. Such antibodies are particularly useful as carriers for drug delivery into a targeted cell.

An example of an antibody possessing all of the aforementioned functional characteristics is D10, which has a heavy chain region encoded by SEQ ID NO: 13 and a light chain region encoded by SEQ ID NO: 15.

In another aspect, polypeptides are provided which consist of or comprise antibodies which specifically bind ROR1 protein and are (a) comprised of a heavy chain region having at least 90% sequence identity with any of the sequences of SEQ. ID. NOs: 2, 6, 10, 14 or 18, (b) comprised of a corresponding light chain region having at least 90% sequence identity with any of the sequences of SEQ ID NOs: 4, 8, 12, 16 or 20, and (c) specifically binds either the 3' end or middle portion of the Ig-like region of the extracellular domain of human or murine ROR-1 protein. In one aspect, the isolated polypeptide is an antibody. In a further aspect, the polypeptide is a Fab or F(ab)'2.

In certain embodiments, an antibody of the present invention may further contain a detectable label. Such labels are known in the art and include radio-isotopes and fluorescent labels. As such, internalization of a compound evidencing passage through transporters can be detected by detecting a signal from within a cell from any of a variety of reporters. The reporter can be a label such as a fluorophore, a chromophore, a radioisotope. Confocal imagining can also be used to detect internalization of a label as it provides sufficient spatial resolution to distinguish between fluorescence on a cell surface and fluorescence within a cell; alternatively, confocal imaging can be used to track the movement of compounds over time. In another approach, internalization of a compound is detected using a reporter that is a substrate for an enzyme expressed within a cell. Once the complex is internalized, the substrate is metabolized by the enzyme and generates an optical signal or radioactive decay that is indicative of uptake. Light emission can be monitored by commercial PMT-based instruments or by CCD-based imaging systems. In addition, assay methods utilizing LCMS detection of the transported compounds or electrophysiological signals indicative of transport activity are also employed.

In certain therapeutic embodiments, the selected antibody may be administered alone, in combination with another antibody of the invention, or with one or more combinatorial therapeutic agents to treat an ROR-1 cancer. When one or more the antibodies described herein are administered as therapeutic agents, they may exert a beneficial effect in the subject by a variety of mechanisms. For example, in certain embodiments, antibodies that specifically bind ROR1 are purified and administered to a patient to neutralize one or more forms of ROR1, to block one or more activities of ROR1, or to block or inhibit an interaction of one or more forms of ROR1 with another biomolecule; e.g., to treat CLL or other ROR1 cancers. All such therapeutic methods are practiced by delivery of a therapeutically effective dosage of a pharmaceutical composition containing the therapeutic antibodies and agents, which can be determined by a pharmacologist or clinician of ordinary skill in human cancer immunotherapy.

In one embodiment, the present invention provides for a method for of treating cancer by the administration to a human subject in need thereof of a therapeutically effective dose of an antibody according to the invention.

In another embodiment, the present invention provides a method for of treating cancer comprising administration to a human subject in need thereof of a therapeutically effective dose of an antibody according to the invention.

Advantageously, the methods of the invention provide for reduction of leukemic or lymphomic cell burden (as demonstrated in and equivalent to an art-accepted animal model) of 2-8 times, or at least 2, 3, 4, 5, 6, 7, or 8 times, that of wild-type human anti-ROR1 antibody or monoclonal 4A5 antibody (disclosed herein).

The methods of the invention further provide a therapeutic approach to inhibiting $CD5^{dull}B220^{+}$ and $ROR1^{bright}B220^{+}$ leukemic B cell expansion.

As discussed herein, the antibodies of the invention may include humanized antibodies, and can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, and optionally with adjunctive or combinatorially active molecules such as anti-inflammatory and anti-fibrinolytic drugs. Antibodies which readily internalize into cells as demonstrated herein with respect to the D10 antibody are also of particular use as carriers for drug delivery into target cells (for example, as shown in FIGS. **21-23**). Those of ordinary skill in the art will be familiar with methods for producing antibody-drug conjugates useful in such drug delivery protocols.

In carrying out various assay, diagnostic, and therapeutic methods of the invention, it is desirable to prepare in advance kits comprises a combination of antibodies as described herein with other materials. For example, in the case of sandwich enzyme immunoassays, kits of the invention may contain an antibody that specifically binds ROR1 optionally linked to an appropriate carrier, a freeze-dried preparation or a solution of an enzyme-labeled monoclonal antibody which can bind to the same antigen together with the monoclonal antibody or of a polyclonal antibody labeled with the enzyme in the same manner, a standard solution of purified ROR1, a buffer solution, a washing solution, pipettes, a reaction container and the like. In addition, the kits optionally include labeling and/or instructional materials providing directions (i.e., protocols) for the practice of the methods described herein in an assay environment. While the instructional materials typically comprise written or printed materials, they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

In general, an in vitro method of diagnosing a ROR-1 cancer will comprise contacting putative cancer cells from a human subject with an antibody according to the invention, and detecting binding with ROR-1 expressed on said cells as compared to expression on post-embryonic human non-cancer cells. All such diagnostic methods are practiced by delivery of a diagnostically effect quantity of antibodies according to the invention, which can be determined by a diagnostician or in vitro diagnostic engineer of ordinary skill in human cancer diagnosis.

The following examples are intended to illustrate but not limit the invention.

EXAMPLE 1

Generation of Monoclonal Anti-ROR1 Antibodies

For the production of the hybridoma-generated mAbs, mice were inoculated with DNA, protein and adenoviral constructs that express the extracellular portion (AA 1-406) of the ROR1 protein that include the Ig-like, CRD and Kringle domains and adjacent linker regions (FIGS. **10-11**). Because of the high degree of homology between the murine and human molecules, a variety of cytokines and immune stimulatory agents, such as Freund's Complete Adjuvant, were co-injected to maximize the generation of anti-human ROR1 antibodies. Hybridoma-generated mAbs were generated and screened for binding to human and murine ROR1. An example of hybridoma derived mAbs is D10.

EXAMPLE 2

Generation of Anti-ROR1 Antibodies Using Phage Display

A second set of antibodies was generated through the use of a proprietary enhanced phage library (Alere, Inc. San Diego). These anti-human ROR1 antibodies bind epitopes that span the entire length of the extra-cellular domain of the ROR1 protein (FIG. **12**). An example of a phage display derived anti-ROR1 antibody is 4A5.

EXAMPLE 3

In Vitro Analysis of Anti-ROR1 Antibodies

Antibodies generated through either hybridomas or phage display were screened for binding to human and murine ROR1. It was determined that the anti-ROR1 antibodies D10 and 4A5 bound only to human ROR1 and did not cross react with murine ROR1.

EXAMPLE 4

Determination of Binding Sites for Anti-ROR1 Antibodies

Because the anti-ROR1 mAbs are species specific, a series of chimeric proteins were generated that were used to determine the binding site for each of the anti-ROR1 mAbs (FIG. **13**). As a second level screen, a series of deletion constructs were generated to determine the actual extracellular ROR1 domain to which the mAbs bind. Once the binding domain was identified, truncated chimeric ROR1 molecules to identify specific sub-regions were generated that are recognized by the anti-human ROR1 mAbs (FIG. **14**). As a final step, the actual amino acids targeted by these antibodies were determined. For this final screen, murinized human amino acids in the sub-domain fragments were generated to determine critical residues required for mAb binding (FIG. **15**). From this screening paradigm, the binding sub-domains for the mAbs were determined (FIG. **15**). It was determine that the D10 anti-human ROR1 mAb required the glutamic acid residue at position 138 for binding to the Ig-like domain of the human ROR1 molecule. When this amino acid is replaced with the murine molecule's lysine residue, the D10 molecule no longer bound to the ROR1 protein.

In a similar manner, it was determined that 4A5 anti-human ROR1 mAb required the isoleucine residue at position 111 for binding to human ROR1 molecule (FIG. **24**). When this amino acid is replaced with the murine molecule's asparagine residue, the 4A5 molecule no longer bound to the ROR1 protein. It was also determined that the anti-ROR1 antibodies G11, H10 and G3 bind the same region as 4A5.

Using standard cross blocking techniques the binding sites for anti-ROR1 antibodies F2, F12 and G6 were determined. These experiments determined that antibodies F2, F12 and G6 cross block the anti-ROR1 antibody D10, indicating that they share a binding site.

EXAMPLE 5

Determination of the K_D Values for the Anti-ROR1 Antibodies D10 and 4A5

The K_D values for the anti-ROR1 antibodies was determined using standard techniques. It was determined that the K_D for the D10 antibody was 40 nM and for the antibody 4A5 was 4 nM (FIGS. **16**A & B).

EXAMPLE 6

In Vivo Analysis of Anti-ROR1 Antibodies

The D10 mAb was assessed in several in vivo models. In a murine in vivo xenograph, niche-dependent, activity model two doses of the mAb were administered at 10 mg/kg against 4 primary patient CLL cells in 76 mice. As shown in FIG. **17**, D10 mAb substantially eliminated patient CLL cells in a dose dependent manner. In contrast, the 4A5 mAb had minimal activity in these studies even though the kDa of this mAb is 10 fold greater (4 vs. 40) for the D10 mAb.

In addition to this activity model, the D10 mAb was also tested in an immune competent transgenic mouse model that spontaneously generates leukemic cells expressing the human ROR1 protein (FIGS. **18-20**). The ROR1-specific mAbs D10 and 4A5 or control IgG antibodies (10 mg/kg) were administered before and after adoptive transfer of ROR1xTCL1 CLL B cells into Balb C mice. The D10 mAb, but not control IgG or 4A5, was able to inhibit the development and expansion of the ROR1xTCL1 leukemic B cells in the blood of recipient animals until two weeks after receiving the last infusion of MAb.

Along with the anti-leukemic activity of this mAb, it has also been shown that the D10 anti-ROR1 antibody is internalized into patient CLL cells and B cell leukemia and lymphoma cell lines at a greater rate and degree than other anti-ROR1 MAbs that bind other antigenic sites on the extracellular portion of the ROR1 protein (FIGS. **21-23**). Because of the absence of the ROR1 protein on post-partum tissues and its rapid rate of internalization, the D10 mAb may serve as an excellent carrier protein for drugs; for example, for use in directed antibody-drug conjugate (ADC) mediated cytotoxicity. Based on these preclinical findings, the D10 mAb has potential to have therapeutic activity against ROR1 expressing leukemias, lymphomas and solid tumor cancers as a targeted therapy and/or conjugated drug carrier.

Although the foregoing subject matter has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be understood by those skilled in the art that certain changes and modifications can be practiced within the scope of the appended claims.

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Gly Arg Phe Thr Ile Ser Arg Asp Asn Val Arg Asn Ile Leu Tyr Leu
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Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
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35 40 45

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Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
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Met Gln Leu Lys Ser Leu Thr Ser Asp Asp Ser Ala Val Tyr Tyr Cys
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<210> SEQ ID NO 13
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 13

caggtgcagc tgaaggagtc aggacctggc ctggtggcgc cctcacagac tctgtccatc 60

acttgcactg tctctgggtt ttcattaacc agttatggtg tacactgggt tcgccagcct 120

ccaggaaagg gtctggagtg gctgggagta atatgggctg gtggattcac aaattataat 180

tcggctctca agtccagact gagcatcagc aaagacaact ccaagagcca agttctctta 240

aaaatgacca gtctgcaaac tgatgacaca gccatgtact actgtgccag gagaggtagt 300

tcctattcta tggactattg gggtcaagga acctcagtca ccgtctcctc a 351

<210> SEQ ID NO 14
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 14

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ala Gly Gly Phe Thr Asn Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Leu Leu
65 70 75 80

Lys Met Thr Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Arg Arg Gly Ser Ser Tyr Ser Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser
115

<210> SEQ ID NO 15
<211> LENGTH: 318
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 15

gaaattgtgc tctctcagtc tccagccatc acagctgcat ctctgggcca aaaggtcacc 60

atcacctgca gtgccagttc aaatgtaagt tacatccact ggtaccagca gaggtcaggc 120

acctccccca gaccatggat ttatgaaata tccaaactgg cttctggagt cccagttcgc 180

ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcagcagcat ggaggctgaa 240

gatgctgcca tttattattg tcagcagtgg aattatcctc ttatcacgtt cggctcgggg 300

acaaagttgg aaatacaa 318

<210> SEQ ID NO 16
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse

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<400> SEQUENCE: 16

Glu Ile Val Leu Ser Gln Ser Pro Ala Ile Thr Ala Ala Ser Leu Gly
1 5 10 15

Gln Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Asn Val Ser Tyr Ile
20 25 30

His Trp Tyr Gln Gln Arg Ser Gly Thr Ser Pro Arg Pro Trp Ile Tyr
35 40 45

Glu Ile Ser Lys Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Ile Tyr Tyr Cys Gln Gln Trp Asn Tyr Pro Leu Ile Thr
85 90 95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Gln
100 105

<210> SEQ ID NO 17
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 17

gaagtgaagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cactttcagt agctatgcca tgtcttgggt tcgccagact 120

ccagagaaga ggctggagtg ggtcgcttcc attagtactg gtgctagcgc ctactttcca 180

gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat cctgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatt attgtgcaag gattactacg 300

tctacctggt acttcgatgt ctggggcgca gggaccacgg tcaccgtctc ctca 354

<210> SEQ ID NO 18
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 18

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Thr Gly Ala Ser Ala Tyr Phe Pro Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
65 70 75 80

Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Arg Ile Thr Thr Ser Thr Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

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<210> SEQ ID NO 19
<211> LENGTH: 322
<212> TYPE: DNA
<213> ORGANISM: Mouse

<400> SEQUENCE: 19

gacatcaaga tgacccagtc tccatcttcc atgtatgcat ctctaggaga gagagtcact 60

atcacttgca aggcgagtca ggacattaat agttatttaa gctggttcca gcagaaacca 120

gggaaatctc ctaagaccct gatctatcgt gcaaacagat tggtagatgg ggtcccatca 180

aggttcagtg gcagtggatc tgggcaagat tattctctca ccatcagcag cctggagtat 240

gaagatatgg gaatttatta ttgtctacag tatgatgagt ttccgtacac gttcggaggg 300

gggaccaagc tggaaataaa ac 322

<210> SEQ ID NO 20
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mouse

<400> SEQUENCE: 20

Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Ala Ser Leu Gly
1 5 10 15

Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
20 25 30

Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile
35 40 45

Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Tyr
65 70 75 80

Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 21
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(62)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 21

Glu Val Lys Leu Val Glu Ser Gly Gly Xaa Gly Leu Val Lys Pro Gly
1 5 10 15

Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Xaa Xaa
20 25 30

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Xaa Xaa Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ile Pro Glu Lys
        35                  40                  45

Arg Leu Glu Trp Val Ala Ser Ile Ser Arg Gly Xaa Xaa Xaa Gly Thr
    50                  55                  60

Thr Tyr Tyr Pro Asp Ser Val Lys Xaa Gly Arg Phe Thr Ile Ser Arg
65                  70                  75                  80

Asp Asn Val Arg Asn Ile Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser
                85                  90                  95

Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
            100                 105


<210> SEQ ID NO 22
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(62)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 22

Glu Val Lys Leu Val Glu Ser Gly Gly Xaa Gly Leu Val Lys Pro Gly
1               5                   10                  15

Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Xaa Xaa
            20                  25                  30

Xaa Xaa Ser Ser Tyr Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys
        35                  40                  45

Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Xaa Xaa Xaa Ser Tyr
    50                  55                  60

Thr Tyr Tyr Pro Asp Ser Val Lys Xaa Gly Arg Phe Thr Ile Ser Arg
65                  70                  75                  80

Asp Asn Ala Arg Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser
                85                  90                  95

Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
            100                 105


<210> SEQ ID NO 23
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 23

Glu Val Gln Leu Val Glu Ser Gly Gly Xaa Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Xaa Xaa
20 25 30

Xaa Xaa Ser Ser Tyr Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys
35 40 45

Gly Leu Glu Trp Val Ser Tyr Ile Ser Ser Ser Xaa Xaa Ser Ser Thr
50 55 60

Ile Tyr Tyr Ala Asp Ser Val Lys Xaa Gly Arg Phe Thr Ile Ser Arg
65 70 75 80

Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala
85 90 95

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 24
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 24

Glu Val Gln Leu Gln Gln Ser Gly Pro Xaa Glu Leu Glu Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ala Phe Xaa Xaa
20 25 30

Xaa Xaa Thr Gly Tyr Asn Met Asn Trp Val Lys Gln Thr Asn Gly Lys
35 40 45

Ser Leu Glu Trp Ile Gly Ser Ile Asp Pro Tyr Xaa Xaa Tyr Gly Gly
50 55 60

Ser Thr Tyr Asn Gln Lys Phe Lys Xaa Asp Lys Ala Thr Leu Thr Val
65 70 75 80

Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser
85 90 95

Asp Asp Ser Ala Val Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 25
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(63)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 25

Glu Phe Gln Leu Gln Gln Ser Gly Pro Xaa Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Xaa Xaa
20 25 30

Xaa Xaa Thr Asp Tyr Asn Met Asn Trp Val Lys Gln Ser Asn Gly Lys
35 40 45

Ser Leu Glu Trp Ile Gly Val Ile Asn Pro Asn Xaa Xaa Xaa Xaa Thr
50 55 60

Thr Ser Tyr Asn Gln Lys Phe Lys Xaa Gly Lys Ala Thr Leu Thr Val
65 70 75 80

Asp Gln Ser Ser Ser Thr Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser
85 90 95

Ser Asp Ser Ala Val Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 26
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 26

Gln Val Gln Leu Val Gln Ser Gly Ala Xaa Glu Val Lys Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Xaa Xaa
20 25 30

Xaa Xaa Thr Gly Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln
35 40 45

Gly Leu Glu Trp Met Gly Trp Ile Asn Pro Asn Xaa Xaa Ser Gly Gly
50 55 60

Thr Asn Tyr Ala Gln Lys Phe Gln Xaa Gly Arg Val Thr Met Thr Arg
65 70 75 80

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Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser
                85                  90                  95

Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg
            100                 105


<210> SEQ ID NO 27
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 27

Gln Val Gln Leu Gln Gln Pro Gly Ala Xaa Glu Leu Val Lys Pro Gly
1               5                   10                  15

Thr Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Asn Phe Xaa Xaa
            20                  25                  30

Xaa Xaa Thr Asn Tyr Trp Ile Asn Trp Val Lys Leu Arg Pro Gly Gln
        35                  40                  45

Gly Leu Glu Trp Ile Gly Glu Ile Tyr Pro Gly Xaa Xaa Ser Gly Ser
    50                  55                  60

Thr Asn Tyr Asn Glu Lys Phe Lys Xaa Ser Lys Ala Thr Leu Thr Ala
65                  70                  75                  80

Asp Thr Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Ala Ser
                85                  90                  95

Glu Asp Ser Ala Leu Tyr Tyr Cys Ala Arg
            100                 105


<210> SEQ ID NO 28
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
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<400> SEQUENCE: 28

Gln Val Gln Leu Gln Gln Pro Gly Ala Xaa Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Xaa Xaa
20 25 30

Xaa Xaa Thr Ser Tyr Trp Ile Thr Trp Val Lys Gln Arg Pro Gly Gln
35 40 45

Gly Leu Glu Trp Ile Gly Asp Ile Tyr Pro Gly Xaa Xaa Ser Gly Ser
50 55 60

Thr Asn Tyr Asn Glu Lys Phe Lys Xaa Ser Lys Ala Thr Leu Thr Val
65 70 75 80

Asp Thr Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser
85 90 95

Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 29
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 29

Gln Val Gln Leu Val Gln Ser Gly Ala Xaa Glu Val Lys Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Xaa Xaa
20 25 30

Xaa Xaa Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln
35 40 45

Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Xaa Xaa Gly Gly Ser
50 55 60

Thr Ser Tyr Ala Gln Lys Phe Gln Xaa Gly Arg Val Thr Met Thr Arg
65 70 75 80

Asp Thr Ser Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser
85 90 95

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 30
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(62)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 30

Glu Val Lys Leu Val Glu Ser Gly Gly Xaa Gly Leu Val Lys Pro Gly
1 5 10 15

Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Xaa Xaa
20 25 30

Xaa Xaa Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys
35 40 45

Arg Leu Glu Trp Val Ala Ser Ile Ser Thr Gly Xaa Xaa Xaa Ala Ser
50 55 60

Thr Tyr Phe Pro Asp Ser Val Lys Xaa Gly Arg Phe Thr Ile Ser Arg
65 70 75 80

Asp Asn Ala Arg Asn Ile Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser
85 90 95

Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 31
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 31

Glu Val Lys Leu Val Glu Ser Gly Gly Xaa Gly Leu Val Lys Pro Gly
1 5 10 15

Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Xaa Xaa
20 25 30

Xaa Xaa Ser Ser Tyr Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys
35 40 45

Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Xaa Xaa Gly Ala Ser
50 55 60

Thr Tyr Tyr Pro Asp Ser Val Lys Xaa Gly Arg Phe Thr Ile Ser Arg
65 70 75 80

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Asp Asn Ala Arg Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser
                85                  90                  95

Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
            100                 105


<210> SEQ ID NO 32
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(61)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 32

Glu Val Gln Leu Val Glu Ser Gly Gly Xaa Gly Leu Val Gln Pro Gly
1               5                   10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Xaa Xaa
            20                  25                  30

Xaa Xaa Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys
        35                  40                  45

Gly Leu Glu Trp Val Ser Ala Ile Ser Gly Ser Xaa Xaa Gly Gly Ser
    50                  55                  60

Thr Tyr Tyr Ala Asp Ser Val Lys Xaa Gly Arg Phe Thr Ile Ser Arg
65                  70                  75                  80

Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala
                85                  90                  95

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
            100                 105


<210> SEQ ID NO 33
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
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<222> LOCATION: (60)..(62)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
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<400> SEQUENCE: 33

Gln Val Gln Leu Lys Glu Ser Gly Pro Xaa Gly Leu Val Ala Pro Ser
1 5 10 15

Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Xaa Xaa
20 25 30

Xaa Xaa Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys
35 40 45

Gly Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Xaa Xaa Xaa Gly Phe
50 55 60

Thr Asn Tyr Asn Ser Ala Leu Lys Xaa Ser Arg Leu Ser Ile Ser Lys
65 70 75 80

Asp Asn Ser Lys Ser Gln Val Leu Leu Lys Met Thr Ser Leu Gln Thr
85 90 95

Asp Asp Thr Ala Met Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 34
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Mouse
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(62)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 34

Gln Val Gln Leu Lys Glu Ser Gly Pro Xaa Gly Leu Val Ala Pro Ser
1 5 10 15

Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Xaa Xaa
20 25 30

Xaa Xaa Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro Pro Gly Lys
35 40 45

Gly Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Xaa Xaa Xaa Gly Ser
50 55 60

Thr Asn Tyr Asn Ser Ala Leu Met Xaa Ser Arg Leu Ser Ile Ser Lys
65 70 75 80

Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr
85 90 95

Asp Asp Thr Ala Met Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 35
<211> LENGTH: 106
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(34)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(62)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (73)..(73)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 35

Gln Val Gln Leu Gln Glu Ser Gly Pro Xaa Gly Leu Val Lys Pro Ser
1 5 10 15

Gln Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Xaa Xaa
20 25 30

Xaa Xaa Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys
35 40 45

Gly Leu Glu Trp Ile Gly Glu Ile Asn His Ser Xaa Xaa Xaa Gly Ser
50 55 60

Thr Asn Tyr Asn Pro Ser Leu Lys Xaa Ser Arg Val Thr Ile Ser Val
65 70 75 80

Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala
85 90 95

Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
100 105

<210> SEQ ID NO 36
<211> LENGTH: 406
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Met His Arg Pro Arg Arg Arg Gly Thr Arg Pro Pro Leu Leu Ala Leu
1 5 10 15

Leu Ala Ala Leu Leu Leu Ala Ala Arg Gly Ala Ala Ala Gln Glu Thr
20 25 30

Glu Leu Ser Val Ser Ala Glu Leu Val Pro Thr Ser Ser Trp Asn Ile
35 40 45

Ser Ser Glu Leu Asn Lys Asp Ser Tyr Leu Thr Leu Asp Glu Pro Met
50 55 60

Asn Asn Ile Thr Thr Ser Leu Gly Gln Thr Ala Glu Leu His Cys Lys
65 70 75 80

Val Ser Gly Asn Pro Pro Pro Thr Ile Arg Trp Phe Lys Asn Asp Ala
85 90 95

Pro Val Val Gln Glu Pro Arg Arg Leu Ser Phe Arg Ser Thr Ile Tyr
100 105 110

Gly Ser Arg Leu Arg Ile Arg Asn Leu Asp Thr Thr Asp Thr Gly Tyr
115 120 125

Phe Gln Cys Val Ala Thr Asn Gly Lys Glu Val Val Ser Ser Thr Gly
130 135 140

Val Leu Phe Val Lys Phe Gly Pro Pro Pro Thr Ala Ser Pro Gly Tyr
145 150 155 160

Ser Asp Glu Tyr Glu Glu Asp Gly Phe Cys Gln Pro Tyr Arg Gly Ile
165 170 175

Ala Cys Ala Arg Phe Ile Gly Asn Arg Thr Val Tyr Met Glu Ser Leu
180 185 190

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His Met Gln Gly Glu Ile Glu Asn Gln Ile Thr Ala Ala Phe Thr Met
        195                 200                 205

Ile Gly Thr Ser Ser His Leu Ser Asp Lys Cys Ser Gln Phe Ala Ile
    210                 215                 220

Pro Ser Leu Cys His Tyr Ala Phe Pro Tyr Cys Asp Glu Thr Ser Ser
225                 230                 235                 240

Val Pro Lys Pro Arg Asp Leu Cys Arg Asp Glu Cys Glu Ile Leu Glu
                245                 250                 255

Asn Val Leu Cys Gln Thr Glu Tyr Ile Phe Ala Arg Ser Asn Pro Met
            260                 265                 270

Ile Leu Met Arg Leu Lys Leu Pro Asn Cys Glu Asp Leu Pro Gln Pro
        275                 280                 285

Glu Ser Pro Glu Ala Ala Asn Cys Ile Arg Ile Gly Ile Pro Met Ala
    290                 295                 300

Asp Pro Ile Asn Lys Asn His Lys Cys Tyr Asn Ser Thr Gly Val Asp
305                 310                 315                 320

Tyr Arg Gly Thr Val Ser Val Thr Lys Ser Gly Arg Gln Cys Gln Pro
                325                 330                 335

Trp Asn Ser Gln Tyr Pro His Thr His Thr Phe Thr Ala Leu Arg Phe
            340                 345                 350

Pro Glu Leu Asn Gly Gly His Ser Tyr Cys Arg Asn Pro Gly Asn Gln
        355                 360                 365

Lys Glu Ala Pro Trp Cys Phe Thr Leu Asp Glu Asn Phe Lys Ser Asp
    370                 375                 380

Leu Cys Asp Ile Pro Ala Cys Asp Ser Lys Asp Ser Lys Glu Lys Asn
385                 390                 395                 400

Lys Met Glu Ile Leu Tyr
                405


<210> SEQ ID NO 37
<211> LENGTH: 406
<212> TYPE: PRT
<213> ORGANISM: Murine

<400> SEQUENCE: 37

Met His Arg Pro Arg Arg Arg Gly Thr Arg Pro Pro Pro Leu Ala Leu
1               5                   10                  15

Leu Ala Ala Leu Leu Leu Ala Ala Arg Gly Ala Asp Ala Gln Glu Thr
            20                  25                  30

Glu Leu Ser Val Ser Ala Glu Leu Val Pro Thr Ser Ser Trp Asn Thr
        35                  40                  45

Ser Ser Glu Ile Asp Lys Gly Ser Tyr Leu Thr Leu Asp Glu Pro Met
    50                  55                  60

Asn Asn Ile Thr Thr Ser Leu Gly Gln Thr Ala Glu Leu His Cys Lys
65                  70                  75                  80

Val Ser Gly Asn Pro Pro Pro Ser Ile Arg Trp Phe Lys Asn Asp Ala
                85                  90                  95

Pro Val Val Gln Glu Pro Arg Arg Ile Ser Phe Arg Ala Thr Asn Tyr
            100                 105                 110

Gly Ser Arg Leu Arg Ile Arg Asn Leu Asp Thr Thr Asp Thr Gly Tyr
        115                 120                 125

Phe Gln Cys Val Ala Thr Asn Gly Lys Lys Val Val Ser Thr Thr Gly
    130                 135                 140

Val Leu Phe Val Lys Phe Gly Pro Pro Pro Thr Ala Ser Pro Gly Ser
145                 150                 155                 160
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Ser Asp Glu Tyr Glu Glu Asp Gly Phe Cys Gln Pro Tyr Arg Gly Ile
                165                 170                 175

Ala Cys Ala Arg Phe Ile Gly Asn Arg Thr Val Tyr Met Glu Ser Leu
            180                 185                 190

His Met Gln Gly Glu Ile Glu Asn Gln Ile Thr Ala Ala Phe Thr Met
        195                 200                 205

Ile Gly Thr Ser Ser His Leu Ser Asp Lys Cys Ser Gln Phe Ala Ile
    210                 215                 220

Pro Ser Leu Cys His Tyr Ala Phe Pro Tyr Cys Asp Glu Thr Ser Ser
225                 230                 235                 240

Val Pro Lys Pro Arg Asp Leu Cys Arg Asp Glu Cys Glu Val Leu Glu
                245                 250                 255

Asn Val Leu Cys Gln Thr Glu Tyr Ile Phe Ala Arg Ser Asn Pro Met
            260                 265                 270

Ile Leu Met Arg Leu Lys Leu Pro Asn Cys Glu Asp Leu Pro Gln Pro
        275                 280                 285

Glu Ser Pro Glu Ala Ala Asn Cys Ile Arg Ile Gly Ile Pro Met Ala
    290                 295                 300

Asp Pro Ile Asn Lys Asn His Lys Cys Tyr Asn Ser Thr Gly Val Asp
305                 310                 315                 320

Tyr Arg Gly Thr Val Ser Val Thr Lys Ser Gly Arg Gln Cys Gln Pro
                325                 330                 335

Trp Asn Ser Gln Tyr Pro His Ser His Thr Phe Thr Ala Leu Arg Phe
            340                 345                 350

Pro Glu Leu Asn Gly Gly His Ser Tyr Cys Arg Asn Pro Gly Asn Gln
        355                 360                 365

Lys Glu Ala Pro Trp Cys Phe Thr Leu Asp Glu Asn Phe Lys Ser Asp
    370                 375                 380

Leu Cys Asp Ile Pro Ala Cys Asp Ser Lys Asp Ser Lys Glu Lys Asn
385                 390                 395                 400

Lys Met Glu Ile Leu Tyr
                405
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What is claimed is:

1. An isolated antibody that specifically binds receptor tyrosine kinase like orphan receptor one (ROR1) protein and comprises a heavy chain variable region comprising SEQ ID NO:14 and a light chain region comprising SEQ ID NO:16.

2. The antibody according to claim **1**, wherein it further specifically binds either the 3' or middle Ig-like region of the extracellular domain of human or murine ROR-1 protein.

3. The antibody according to claim **1**, wherein it binds the 3' end of the Ig-like region of the extracellular domain of human or murine ROR-1 protein from position 1-147.

4. The antibody according to claim **3**, wherein it further binds a glutamic acid residue corresponding to the one found in the extracellular domain of human ROR-1 protein at position 138.

5. The antibody according to claim **1**, wherein it further reduces leukemic or lymphomic cell burden in an art-accepted animal model at a rate of 2-8 times, or at least 2, 3, 4, 5, 6, 7, or 8 times that of wild-type human anti-ROR1 antibody or monoclonal 4A5 antibody.

6. The antibody according to claim **1**, wherein it further inhibits $CD5^{dull}$ $B220^{+}$and $ROR1^{bright}$ $B220^{+}$leukemic B cell expansion.

7. The antibody according to claim **1**, wherein it further is internalized into leukemic or lymphomic cells at a rate of at least 2 times, or at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 times that of monoclonal antibody 4A5.

8. A pharmaceutically acceptable anti-ROR1 antibody composition comprising the antibody according to claim **1** and a pharmaceutically acceptable carrier.

9. An isolated antibody which binds the same epitope as antibody D10, wherein the heavy chain variable region of D10 is encoded by SEQ ID NO:13 and the light chain variable region of D10 is encoded by SEQ ID NO:15.

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